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The Journal of Laboratory and Clinical Medicine

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ORIGINAL ARTICLES

A STUDY OF THE LIPIN CONTENT OF THE LIVER IN TWO CASES OF DYSPITUITARISM*

BY ALDRED SCOTT WARTHIN, PH.D., M.D., ANN ARBOR, MICH.

THE question of the fat-content of the various organs and tissues, in normal and in pathologic conditions, has been the subject of much discussion and research during the last decade. As the result of numerous investigations we know today that the majority of the tissues and all of the organs of the body contain fat under normal conditions. As regards the presence of fat normally in the hematopoietic organs there is still some conflict of opinion; but in certain pathological conditions these organs show marked accumulations of lipoid bodies showing that they must bear some important relation to certain forms or phases of fat-metabolism, at least. Great interest has been aroused by the discovery of the important part played by the lipoid bodies in various pathologic processes, and lipoid cells (cells with large amount of pale, vacuolated, foamy, reticulated cytoplasm and small, deeply staining eccentrically placed nuclei) are at the present time attracting much attention in pathologic histology. They have been found in many pathological processes; in inflammatory infiltrations, particularly lymphoid areas, in chronic aortitis, appendicitis, salpingitis, pyelitis, old ovarian abscesses, chronic mastitis, chronic cholangitis, prostatitis, chronic nephritis, actinomycotic abscesses, chronic granulation tissue, leprosy, rhinoscleroma, chorioid plexus, adrenals, neoplasms, etc. The xanthoma and pseudoxanthoma cells are lipoid cells. As to the histologic origin of the lipoid cells there has been much discussion; they are probably fibroblastic (reticulo-endothelial) cells in a state of lipoid infiltration (lipoid macrophages).

The chemical nature of the lipoid bodies represents still an almost unknown field. The school of Aschoff divides them into three groups, *glycerin-esters, cholesterin-esters,* and *lipoids* in a narrower sense (phosphatid, cerebrosid, fatty acids, and soaps). Mixtures of these substances, particularly of cholesterinesters

*From the Pathological Laboratory of the University of Michigan.

and lipoids, are not rare. Histologically the lipoids may be demonstrated by staining with osmic acid, Sudan III, Scharlach R, Neutral red, Nile blue; by the methods of Smith-Dietrich, Fischler, and Ciaccio, and by polarized light. The cholesterol lipoids are doubly refractive (anisotropic) and stain less black with osmic acid, and more yellow or brownish with Sudan III and Scarlet R than do the glycerin-esters.

Aschoff regards cholesterol-ester infiltration as a sign of chronic inflammation or of a chronic disturbance of metabolism. According to his views, in conditions, such as glycosuria, pentosuria, and chronic icterus, in which cholesterin-esters are present in abundance in the blood-plasma (cholesterinæmia) the cholesterin-esters are taken up by the cells (anisotropic fatty infiltration). However, Aschoff's views are not generally accepted, and by some writers are regarded as premature.

Our conceptions of the lipin metabolism of the body have recently been greatly widened by the discovery that lipoid bodies accumulate in the spleen and blood-forming organs in certain pathologic conditions, notably diabetic lipemia and Gaucher's disease. In 1903, Fischer noted in a case of lipemia that the spleen was hyperplastic and showed a diffuse infiltration of fat-droplets. Not until 1912 was a similar observation recorded when W. H. Schultze reported a case of diabetic lipemia in which the spleen showed almost complete replacement of the pulp with large, pale, vacuolated cells containing a lipoid substance probably closely related to cholesterol. Comparing his material with that from two cases of Gaucher's disease he concluded that the pale vacuolated cells in the latter affection were also filled with a lipoid substance and identical with the cells of his case. Brill and Mandlebaum, in 1913, were unable to demonstrate the presence of lipoids in the spleen in a case of Gaucher's disease, but their examination does not appear to have been complete. Lutz, in 1914, reported two cases of large-celled hyperplasia of the spleen pulp in diabetic lipemia, showing that his cases were similar to the one of Schultze; and that the large, pale, vacuolated cells were identical with those seen in the spleen in Gaucher's disease, but that the identity of the lipoid-containing cells did not prove that the two processes were identical. Sapegno has also reported two cases of Gaucher's disease, concluding that the characteristic large pale cells show a deposit of lipoids, and are similar to the cells found in the spleen in diabetic lipemia. He also refers to a case of obstructive jaundice observed by Kramer in which masses of lipoid cells were found in Glisson's capsule of the liver and in the splenic trabeculæ.

Following these observations Anitschkow produced in rabbits by prolonged feeding of cholesterol the same picture of large pale vacuolated cells (cholesterol steatosis) in the spleen, lymph nodes and bone marrow as that seen in Gaucher's disease and diabetic lipemia. In 1916, Wahl and Richardson reported a study of Gaucher's disease in an infant eleven months old. The spleen, liver, and lymph nodes presented the usual picture of Gaucher's disease, but in addition the medulla of both adrenals was almost completely replaced by clusters of large pale vacuolated cells. Similar cells were found in the intestines, Peyer's patches, thymus, and adventitia of some of the smaller vessels. A thorough chemical and histological study was made of this case; and the writers con-

clude that Gaucher's disease is due to a disturbance of lipoid and fat metabolism resulting in the accumulation of lipoid substances in the cytoplasm of large pale cells that are chiefly reticulo-endothelial cells of the spleen, lymph-nodes, bone marrow, liver, Kupffer stellate cells, etc. These cells are concerned in the metabolism of the fats and lipoids and comprise the "endothelial-Stoffwechsel-apparate." In advanced cases specific parenchymal cells also take up the lipoids. There is not only a marked increase in the lipin content, but a marked alteration in the normal relations of the lipins. The fixed fats are greatly reduced, and the lipoids, such as lecithin and cholesterol, greatly increased. A lecithin-like body predominated in their case. Gaucher's disease therefore must be classed with the group of xanthelasmic conditions which are characterized by a more or less diffuse accumulation of lipoids in reticulo-endothelial or fibroblastic cells in one or more organs. It is a more diffuse and widespread involvement of the "endothelial-Stoffwechselapparate" than the cases of large-celled hyperplasia of the spleen seen in diabetic lipemia.

A clinical and pathological study of two cases of Gaucher's disease in infants reported by Knox, Wahl and Schmeisser, in 1916, includes the case given above and the sister of that case, aged fifteen months. The pathological findings in the second case were similar to those of the first; the essential feature being the occurrence, particularly in the hematopoietic system, of large pale vacuolated cells containing a peculiar refractive substance having the chemical and staining properties of lipoid material. These writers regard their two cases as identical with the cases reported as Gaucher's disease, and conclude that this affection "is not primarily a disease of the spleen, or of any other organ, or set of organs, but is a generalized process due to a disturbance in fat metabolism, manifesting itself by lipoid metamorphosis, that is, by the more or less diffuse accumulation of lipoid material in many cells with the formation of the characteristic large pale cells. This process is most prominent in the hematopoietic system, especially in the spleen and lymph nodes, organs that are said to play a not unimportant role in abnormal fat metabolism."

If these conclusions as to the nature of Gaucher's disease are correct, an entirely new field of pathologic metabolism has been opened up, aside from the elucidation of the nature of the mysterious large pale cell characteristic of this process. As our knowledge of the lipoid bodies is so incomplete any new light thrown upon fat and lipoid metabolism becomes particularly valuable at this stage. Through the study of such pathologic disturbances of lipoid metabolism we may gain important knowledge of the normal metabolism. Some progress into this field has already been made. The part played by the adrenals and spleen in lipoid metabolism has already been pointed out by several writers. Krylow believes that the adrenal cortex regulates lipoid metabolism, and that any insufficiency on its part would lead to an accumulation of lipoids in other parts of the body. Chalatow, Soper, Kusunoki, and others have pointed out the importance of the role of the spleen in lipoid metabolism. Soper holds that the spleen's apparent large part in this is due to its extensive reticulo-endothelial system. The liver also plays a very important part in the regulation of cholesterin metabolism. By some writers it is placed between the adrenals and the spleen and endothelial apparatus in its relative importance in so far as this function is concerned. Other organs and

tissues (sex-glands, panniculus, etc.) may play an important role in the maintenance of cholesterol equilibrium, but we as yet have no definite knowledge of such metabolic processes. Increase of cholesterin in the blood has been noted in pregnancy. Injection of cholesterin emulsions in tumor-grafted rats has appeared to increase the rate of tumor-growth (Burnett and Robertson). Luden has found high cholesterin values in patients suffering from malignant disease, and on very insufficient grounds has advanced the hypothesis that cholesterin retention due to insufficient conversion or deficient elimination may be a primary factor in the etiology of malignant disease.

All forms of disturbances of fat metabolism should be studied in the attempt to add knowledge to this subject, particularly those of a generalized character. I wish to present here such a study in a field as yet untouched, the marked disturbances of fat-metabolism occurring in hypophysis disease (dyspituitarism). Two autopsy cases of this condition showing the two stages of this affection, one of adolescent dystrophia adiposo-genitalis, the other of dyspituitarism in an adult following an acromegalic stage, have occurred in my service Both cases presented the striking obesity characterizing hypopituitarism. As far as I have been able to discover no studies have as yet been made of the character of the fat in this condition. Cushing, in his book on "The Pituitary and its Disorders" remarks (page 258) that "The adiposity of hypopituitarism is a generalized one—not limited solely to the panniculus—though its bedside recognition is necessarily restricted to the character of the subcutaneous disposal. The fat shows postmortem, certain peculiarities of color and consistency which suggest a different chemical composition from 'normal panniculus,' and is worthy of a differential analysis. It furthermore invades the organs, and in the liver particularly, there is often an extraordinary replacement of the cells by fat globules." In his description of the changes in the adrenals (page 281) he says: "As is true of the experimental states of hyperpituitarism [?], so in the clinical cases the adrenals which we have had an opportunity of studying histologically have shown an abnormal vacuolization (a lipoid change?) of the cells of the zona fasciculata of the cortex (Fig. 313)." In the legend of this figure the condition is correctly stated as hypopituitarism, evidently a typographical error in the text, and the adrenal pictured shows a condition of marked liposis.

CASE I.

H. F., Schoolboy, aged 18 years. American. Admitted to Neurologic Clinic (Dr. Camp), 1-16-12.

CHIEF COMPLAINT.—Failing vision.

FAMILY HISTORY.—Negative. Two brothers and two sisters, living and well.

PREVIOUS PERSONAL HISTORY.—Has had measles and smallpox. No history of injury. No bad habits. Has had some headaches ever since he can remember, accompanied by nausea and occasional vomiting.

PRESENT TROUBLE.—About one year ago the headaches became more severe and constant. He then had convulsions, and was unconscious for two weeks (with spasms during first week). No fever. Eyes began to fail two years ago. Since the attack of unconsciousness his headaches have been less severe. At present he complains chiefly of a difficulty in vision and constant sleepiness. Is in the 11th grade at school. Seems as bright mentally as the other pupils.

PHYSICAL EXAMINATION.—Very obese boy. Height 4 ft., 10½ in. Weight, 120¾ lbs. Panniculus hangs in long folds from breast and abdomen. Feminine type. Well developed mammæ. Face rounded and full. Skin of good color. No axillary hair; very little pubic hair. Neck very short with adipose collar. Thyroid not enlarged. Genitalia very poorly developed, hardly visible. Right testicle undescended. Hands and feet short and stubby. No enlarged glands. Heart sounds very faint and irregular. Mentality good. Facial expression drowsy. Occasionally a choreiform movement in the extremities. Can count fingers at about five feet. Reflexes diminished. Prickly sensations at times over the skin, particularly in thighs. Urine negative. R.b.c., 4,370,000; w.b.c., 12,050; hemoglobin 90. X-ray examination shows a dense mass about the size of a hazel-nut in the anterior portion of the sella. Ophthalmologic examination showed well-advanced optic atrophy. Blood-pressure 105.

Operated by Dr. Canfield 2-9-12. Took anesthetic badly. Route through the sphenoidal cells removing part of the floor just anterior to the anterior wall of the sella. Through the opening meninges and brain bulged. This was punctured and about two drams of blood and fluid escaped. Operation closed without removal of hypophyseal tumor. Patient recovered consciousness, and remained so for several hours without pain; then became delirious, with spasms of eyes and face, great restlessness. Temperature per rectum 104°. Became gradually semi-conscious. No paralysis. No pain. Developed spasms before death at 6 A. M. next morning. The autopsy was made five hours after death.

AUTOPSY PROTOCOL.—EXTERNAL EXAMINATION.—Body of a very obese boy, apparently about ten to twelve years old. Length 150 cm. Somewhat feminine in type. Breasts very fat; no fluid can be expressed from them. Hands and feet short and thick. Abdomen above level of thorax, large folds of panniculus. Face has a rather stupid adenoid expression. Very fat and rounded. Marked fatty collar. Hair of head is abundant, but there are few axillary and pubic hairs; these are fine and light in color. Both testicles are in the small scrotum; they are about the size of those of a ten year old boy. Right one is smaller. Short retracted foreskin; glans normal. Mucoud secretion dripping from meatus. Panniculus throughout very thick and firm. No edema except slight over ankles. Muscles poorly developed. Rigor mortis only in muscles of jaw. Body is very warm, feels hot to the touch. Teeth negative. Nostrils are dilated, and contain clots of blood. Arms and legs show a number of bruises. Hypostasis is marked, purplish red. Sacrococcygeal dimple is very marked. Percussion of thymus region shows an increased area of dullness.

Spinal Cord.—Not removed.

Brain.—Scalp and periosteum negative. Skull-cap is thin, particularly over the vertex where there are well defined areas of deficient ossification, and also in the region of the fontanels. Dura is firmly adherent all over, and increased in thickness along the longitudinal sinus. Intracerebral pressure greatly increased. Meningeal and pial vessels are markedly congested. Meninges over frontal lobes are covered with fresh blood-clot; and there is an extravasation of blood through the subarachnoid space. On lifting the frontal lobes there is seen a large recent clot between the base and the optic nerves. Blood clots in left temporal and occipital regions. Brain is adherent to the cranial floor in the neighborhood of the

hypophysis. On separating the attachments the softened brain substance in the neighborhood is much torn. It is discolored and partly necrotic and hemorrhagic. A large clot extends between the frontal lobes along the left anterior cerebral. The softening of the brain substance and hemorrhage are most marked in the neighborhood of the chiasm where the basal dura is torn and the floor of the skull penetrated by the operation anterior to the hypophysis. No trace of this organ is visible. The sella is not greatly enlarged and is filled with a projecting mulberry mass, very hard and giving a clear ringing sound when struck. In the floor of the cranium just anterior to the sella is a similar hard nodule the size of a peppercorn. From the hard mass in the sella an atypical softer tissue infiltrates the discolored and softened brain tissue lying above it. The basal arachnoid and pia are much thickened and covered with recent clots. Ventricles dilated, contain a blood-stained fluid. Pineal gland normal in size and appearance. Choroid plexus negative. Cerebral and cerebellar tissues soft, edematous and congested.

Main Section.—Abdominal panniculus 5 cm. thick; that over thorax is 3 cm. It differs in appearance from normal fat; when first cut into it is very glistening, translucent, very firm and is light buffy-yellow in color. The panniculus is very warm to the touch, steaming. On cooling it becomes more buff in color, opaque and very hard. The lobules are much larger than normal panniculus. The appearances suggest a myxedematous fat. The subserous panniculus is 3 cm. thick and of the same character. Omentum and epiploic appendages, mesentery and subserosa of bladder show similar thick deposit of fat. The abdominal and thoracic muscles are small and show abundant fatty infiltrations. No free fluid or gas in peritoneal cavity. Liver very large extending 3 f.b. below ensiform, and 2 f.b. below edge of ribs in right nipple line. Spleen not enlarged. Diaphragm 5th rib on left; 4th on right.

Thorax.—No fluid or gas in thoracic cavity. Lungs free. Apex of heart in 4th intercostal space, left parasternal line. Mediastinal fat very abundant. Thymic region filled with a large pink, two-lobed thymus, the left one extending over left ventricle to apex, the right one covering the right auricle. The upper boundary of the lobes touches the thyroid. Pericardium normal. Heart normal size; undersized if amount of panniculus is taken into account. This is very abundant. Right ventricle contains large red and white clot. Left ventricular wall is 10 mm. thick. Mitral flaps and orifice negative. Pulmonary artery and aorta negative. Foramen ovale and ductus arteriosus closed. The lungs show marked congestion and numerous small hemorrhages. The intima of the ascending portion of the aorta shows patches of fatty degeneration.

Neck Organs.—Thyroid is small. On section shows but little colloid, resembles foetal thyroid. Homogeneous surface. Four brownish-pink parathyroids. Not enlarged. Cervical glands not enlarged.

Abdomen.—Spleen weighs 50 gms. Soft. Capsule negative. Follicles numerous, large. Cut surface shagreened. Large intestine distended; unusually large; otherwise negative. Stomach negative. Pancreas normal in size and consistency. Much fat about it. Both adrenals are large, absolutely and in proportion to size of kidneys. Cortex yellow, medulla hypoplastic, much fat about both adrenals. Kidneys have enormous fatty capsules. Fibrous capsules strip easily. Fetal lobulations well preserved. On section kidneys show

marked congestion. Pelvic fat much increased. Liver weight 1030 gm., capsule negative. Surface mottled, yellowish and red, with fatty shine. Lobules not distinct. Mesentery very fat. Mesenteric and retroperitoneal nodes not enlarged.

Genitalia.—The testes are surrounded by a fatty sheath. On section they appear as undeveloped infantile testes. Prostate cannot be felt or seen. Seminal vesicles not developed. Other organs negative.

Microscopical Examination.—*Tumor of Hypophysis Region.*—Presents the appearance of an adamantino-carcinoma with hyaline and calcareous masses of a dentine-like substance arranged in mulberry masses or agglomerations of rounded concretions. These are formed by the hyaline transformation of cords and strands of large squamous prickle-cells. Cords of these cells infiltrate the brain substance after the manner of a carcinoma; those in the brain for the greater part showed no calcification, and the cell-cords consists chiefly of a central hyaline mass around which cells with very large intercellular bridges or prickles are grouped showing the characteristic palisade arrangement at the periphery of the hyaline mass. Small cords show no hyaline change. The brain tissue about the tumor-cords shows atrophy with marked gliosis, and occasional cellular infiltration. A small area of hypophysis tissue corresponding in structure to the pons anterior was found showing the same adamantinoma infiltration.

Thyroid.—Many follicles contain a thin colloid; but the majority contain none. The organ closely resembles the fetal thyroid.

Parathyroids.—These were hypoplastic and showed fatty infiltration.

Thymus.—Hyperplasia of the medulla; atrophy of cortex.

Heart.—Fatty infiltration.

Aorta.—Diffuse fatty degeneration of the intima, with small raised patches of lipoid cells resembling those of Gaucher's disease.

Lungs.—Marked congestion and edema; hemorrhages; acute purulent bronchitis and bronchopneumonia.

Spleen.—Marked congestion. Large follicles with lymphoid exhaustion. Sclerotic arterioles. Scattered lipoid cells, not numerous.

Stomach.—Negative.

Intestines.—Excessive mucus formation.

Pancreas.—No changes noted.

Mesenteric and Retroperitoneal Nodes.—Lymphoid exhaustion.

Adrenals.—Abundant chromaffinic cells. Hypoplastic medulla; cortex hypertrophic with marked but irregular liposis, involving in some regions the glomerular zone, in others the fascicular. Marked hypertrophy of some of the fascicular cords.

Kidneys.—Cloudy swelling and congestion. Numerous glomerular scars. Scattered lipoid cells in lymphatics about large veins.

Testes.—Infantile in type, with vacuolation of germinal cells. Interstitial cells hypoplastic.

Liver and Panniculus.—Will be described below with that of Case II.

Pathological Diagnosis.—Infantilism, dyspituitarism adiposo-genitalis; adamantino-carcinoma of hypophysis; hyperplastic thymus; lymphatic constitution; general lipomatosis (liposis) most marked in liver (cholesterol steatosis); intraperipheral zonal necrosis of liver; hypoplasia of thyroid and adrenals; post-

operative hemorrhage and operative trauma at base of brain; acute purulent bronchopneumonia (aspiration).

CASE II.

J. B. M., age 29 years; sailor by occupation. By birth American. Admitted to Neurologic Clinic (Dr. Camp), March 9, 1912.

CHIEF COMPLAINT.—Disturbances of vision, staggering, mental disturbances.

FAMILY HISTORY.—Negative. No case of tuberculosis, cancer, or nervous disease.

PERSONAL HISTORY.—Always well until present trouble began. Mentality always good. Has lived an out-of-doors life. Three years ago began to have violent headaches every few days, lasting 1-2 days and followed by vomiting. After two years these stopped suddenly. At this time he began to complain "of not feeling well," but had no well-defined symptoms. Slept a great deal, all night and during the day except when wakened for meals. Would fall asleep on duty. Suddenly noticed a peculiar tendency to lean to the left when walking, as well as a peculiar manner of carrying left arm. Developed hoarseness and a hesitating slow speech, with difficulty in making himself understood. Lost interest in everything, and avoided particularly reaching for things, asking others to do this for him. Lost his skill in marksmanship and his interest in sports. The difficulty in walking has steadily increased, as have choking spells induced by eating. His sister has noticed nothing strange about his mentality except a slight childishness. During the last year he has had five attacks of dyspnea, hard chills, hands drawn up, side of face drawn up, and fever running as high as 103.5°, followed by vomiting. Cannot walk well after attacks, and has fallen several times. At the present time he has to be dressed and fed. Cannot walk out alone. Smokes excessively; denies alcoholism and venereal disease. No note made of sexual state. Last May was examined by Dr. Parker in Detroit who found ½ vision in each eye. Was x-rayed at this time.

PHYSICAL EXAMINATION.—(March 11, 1912, by Dr. Camp). Large build, well nourished, wears a No. 8 shoe. Hands are large, but patient says they have always been so. Head is fairly large; normal shape. No acromegalic jaw. Thyroid not enlarged. Mentality seems fairly clear. Tendency to senseless jokes. Slight clouding of consciousness; memory is defective except for more recent events. No delusions; no mental retardation. Replies are prompt, although speech is slow. Left pupil reacts to light; right does not. Neither pupil reacts to accommodation. Some defect in lateral motion, particularly to the right. Eyeballs do not converge. Temporal hemianopsia in left eye; in the right eye some contraction of the visual fields in all directions, but no distinct hemianopsia. Palpebral fissure a little wider on left side than on right. Right side of face drawn up distinctly better than left. Tongue straight, no tremor, no atrophy. No ataxia; no intention tremor; no hemiasynergia in hands. Slight staggering gait with eyes closed. Sensory tests normal. Reflexes prompt and equal. Examination of heart, lungs and abdominal organs negative. Urine negative. Lumbar juncture negative. Wassermann negative. Patient was examined again on June 29, 1912, by Dr. Camp. Condition about the same. Returned December 2, 1912. Examination by Dr. Camp. Had severe headaches after leaving the hospital in June;

none until yesterday when he had a severe attack with dizziness. Fell heavily, but was not unconscious. Says that he was paralyzed on the left side four months previously. Symptoms about the same as before. Blood and spinal fluid gave negative Wassermann. Headaches increased. Patient feels "all in." Examination by Dr. Canfield revealed the presence of a nasal tumor. Operated on December 21 by Dr. Canfield. Submucous resection of the septum showed a tumor mass extending from the sphenoid cavities from regions back of the sphenoid down into the septum. The tumor was very soft, dark grayish in color, and flecked with white spots. Microscopical examination of this in our laboratory showed the neoplasm to be a large round cell sarcoma containing numerous calcareous concretions (psammoma). Regarded as probably primary in basal meninges.

After the operation condition continued unchanged (headache, sleepiness, etc.). Temperature rose to 105° the day after the operation. On January 16th he complained of more or less constant pain through temples. Seen at intervals of several weeks. Irrational at times. By April 9, 1913, enlargement of hands was notable. His sister said that this had occurred during the last few weeks. Sleepiness increased. By May 19 his face and head looked distinctly larger; appearance of hands typically acromegalic. Feet not grossly enlarged. Chief complaint still headache and "sour stomach." Anxious to have another operation because of "untold agony" in head. Glucose (100 gms.) test negative. Acromegalic condition increased, slowly until January, 1914, when his sister noticed that his shoes were looser. Complained at this time of chilliness; skin of hands mottled and discolored. Tonic convulsive movements in upper extremities. Temperature rises to about 103° after chill. Subcutaneous panniculus all over the body increased. Secondary type of optic atrophy in right eye. Fundus of left is more congested than right. Headaches have ceased. No note of sexual state occurs in the history. Patient showed gradual cessation and subsidence of the acromegalic condition with increasing symptoms of hypopituitarism (increase of panniculus, etc.) until his death on April 15, 1914, at 7:50 A. M. Autopsy at 10 A. M.

AUTOPSY PROTOCOL.—Body large; masculine type; length 175 cm. Acromegalic facies and hands. Obese; panniculus abundant all over body. Body and head hair abundant. Genitalia well-developed; testicles soft and flabby. Body very warm; axillary temperature 99+, rectal 104°. No rigor mortis. Moderate purplish hypostasis.

Spinal Cord.—Not examined.

Head.—Scalp and periosteum negative. Skull-cap thickened; inner surface rough, gray, meningeal grooves prominent. No exostoses. Pacchionian depressions shallow. Diplœ sclerosed. Dura not thickened but adherent throughout. No osteomata in dura.

On lifting and removing the brain a large soft tumor is found at the base, in the interpeduncular space, extending more into the base of the right cerebral hemisphere than on the left. Smaller lobulated masses are found about the periphery of the larger mass. The tumor has destroyed the right optic tract and displaces the 5th and succeeding cranial nerves backward. The third nerve appeared to pass through the tumor. The pons was compressed by the tumor to half its normal thickness. On the right the growth extended into the right temporosphe-

noidal lobe by two finger-like projections, one in the region of the right lenticular body, the other into the third ventricle. The internal capsule was compressed between them. Tumor was reddish, soft, well encapsulated toward the brain substance, but ragged and torn below where it had been separated from the tumor mass extending through the floor of the skull into the retropharynx. Anterior to the sella there was an opening about the size of a silver dollar through which a tumor-mass about the size of a tennis ball extended into the nasopharynx. Base of skull about the opening was soft, necrotic and infiltrated with extensions of the growth. The anterior portion of the sella was destroyed, and no trace of hypophysis tissue could be made out, the entire organ apparently having been destroyed by the growth.

Main Section.—Abdominal panniculus is 5 cm. thick, thoracic 3 cm. The fat is in large coarse lobules, unlike normal fat, pale lemon-buff in color, firm, translucent and shining; but becoming darker and opaque on cooling. The panniculus is very warm to the touch. Omentum is rich in fat of the same type; likewise the epiploic appendages and mesentery. Abdominal organs very hot, almost bloodless. Intra-abdominal temperature 107.1°. Tissues dry very quickly on exposure to air. No free fluid in cavity. Lower border of liver at ensiform in mid-line, a hand's breadth above the lower edge in right nipple line. Slight rigor mortis in abdominal muscles. Diaphragm at 5th ribs.

Thorax.—No fluid in thorax. Apex in left parasternal line behind 5th rib. Lungs meet anteriorly above. No pleural adhesions. Fat tissue in anterior mediastinum very abundant, of same appearance as the panniculus. Large lobules of thymic fat in which remains of thymus are present. On section thymic fat is diffusely pink. Heart is negative, save for a dilatation of the right side, an adherent thrombus in the right auricular appendage, increased subpericardial fat, brown atrophy of muscle and a thickening of the endocardium of the left auricle. Aorta showed early sclerosis in the ascending part of the arch. Lungs show passive congestion. Entire lower lobe of right lung is airless, solid, early stage of red hepatization.

Mouth and Neck.—Hyperkeratosis of tongue (brown hairy tongue). Papillæ at root hyperplastic. Larynx, trachea and esophagus negative. Thyroid is slightly larger than normal, colloid is not diminished. Parathyroids hyperplastic, 8 mm. in diameter, purplish in color. Cervical hemolymph nodes hyperplastic.

Abdomen.—Spleen weighs 120 gms. Small, soft, shows few follicles on section.

Stomach and Intestines.—Dilated, otherwise negative.

Pancreas.—Fatty infiltration of tail. Soft.

Liver.—Very hot to the touch. Clinical thermometer inserted showed five hours after death a temperature of 108°. Small in size, weighs 1730 gms.; sharp edges; mottled surface; yellowish red; opaque fatty shine.

Mesentery is very rich in fat, arrayed in coarse lobules, lemon-yellow color, appearance same as that of panniculi. Fat changes color on exposure to air, becoming a diffuse pink in many areas, giving it a peculiar mottled appearance.

Adrenals are very large, 8 cm. long, 5 cm. broad, and 4 mm. thick. Surrounded by dense masses of fat and old fibrous adhesions.

Kidneys have very large fatty capsules. Fibrous capsule strips easily on the

left, somewhat adherent on the right. On section kidneys show slight atrophy and congestion. Retroperitoneal lymph nodes atrophic, show fatty infiltration. Thoracic and abdominal muscles show fatty infiltrations.

Pelvis.—Penis negative. Left testis rather pale. No increase of stroma visible to the naked eye. Right testis pale, soft. Prostate smaller than normal, congested. Seminal vesicles very much dilated with a thin watery fluid. Walls negative. Bladder and rectum negative.

Microscopical Examination.—Sections of neoplasm from base of brain and nasopharynx show it to be a round cell angiosarcoma with numerous psammoma concretions scattered through it. It bears no resemblance to the hypophyseal struma. In the sella turcica region there were found areas in a number of sections of a typical hypophysis adenoma or struma (chromophobe type) infiltrated, compressed and destroyed by the sarcoma. The appearances lead me to believe that a typical acromegalic hypophyseal struma has been either the seat of a malignant change, or what is more likely has been destroyed for the greater part by a sarcoma arising primarily in the basal meninges or in the floor of the skull. The penetration of the latter, the early growth downward into the nasopharynx and septum support the latter view. The neighboring brain structures show pressure atrophy and necrosis, gliosis and sarcoma infiltrations.

Tongue.—Hyperkeratosis.

Thyroid.—Colloid hyperplasia.

Parathyroids.—Hyperplasia.

Cervical Lymph Nodes.—Marked lymphoid hyperplasia and congestion. Large germ centers with exhaustion of the lymphocytes. Sinuses crowded with macrophages, many of these are hemophages containing red cells or pigment, many of them are filled with fine fat droplets (lipoid cells). Apparent very active hemolysis.

Lungs.—Acute hypostatic pneumonia. Edema and chronic passive congestion.

Heart.—Brown atrophy, fibrosis, fatty infiltration and degeneration; slight cloudy swelling.

Thymus.—Remains of thymus in fat tissue showing newly-formed cords of lymphoid tissue along lymphatics distended with macrophages and hemophages. Hassall's corpuscles small and few.

Spleen.—Chronic passive congestion, atrophy. Few scattered lipoid cells. Numerous hemophages.

Stomach and Intestines.—Acute catarrh. Appendix contains fecal concretion. Old appendicitis.

Pancreas.—Fatty infiltration. Very large fat cells with greenish coloration, showing mixture of lipoids. Hypertrophic islands of Langerhans. Hyaline substance in many acini.

Liver.—See below.

Adrenals.—Marked hypoplasia of medulla. Small amount of chromaffinic tissue. Practically the entire cortex shows a marked liposis of the parenchymatous cells. The liposis is most marked in the glomerular and fascicular zones. Hypertrophic fascicular cords are also numerous. (See further below.)

Kidneys.—Cloudy swelling and fatty degeneration of the tubular epithelium.

Areas of chronic and subacute nephritis. Numerous casts. Lipoidosis of tubular cells.

Aorta.—Early sclerosis. Fatty degeneration of intima. Small elevations made up of collections of lipoid cells in intima.

Mesenteric and Retroperitoneal Lymph Nodes.—Hyperplasia, lymphoid exhaustion, sinus catarrh, great numbers of macrophages and hemophages. New formation of lymphoid tissue throughout mesenteric and retroperitoneal fat. Many lipoid cells.

Hemolymph Nodes.—Hyperplasia. Sinuses crowded with hemophages containing blood cells and pigment. Scattered lipoid cells.

Testes.—Aspermatogenesis. Stroma increased. Basement membranes thickened. Fatty degeneration (liposis) of germinal epithelium.

Prostate.—Moderate glandular hyperplasia.

Seminal Vesicles.—Negative.

PATHOLOGICAL DIAGNOSIS.—Hypopituitarism following acromegaly (sarcoma replacing hypophysis struma); sarcomatous infiltration of brain, base of skull, nasal and pharyngeal cavities; general obesity (general liposis); cholesterol steatosis of liver and adrenals; intraperipheral zonal liver-necroses; postmortem hyperpyrexia; hyperplasia of adrenal cortex, hypoplasia of medulla; hyperplasia of lymphatic and hemolymph nodes with hemolysis; hypertrophy of islands of Langerhans; persistent thymus; lymphoid exhaustion of germ-centers; subacute parenchymatous degenerative nephritis; acute hypostatic pneumonia; acute gastrointestinal catarrh; hyperkeratosis of tongue.

ANALYSIS OF CASES.

These two cases of hypophysis disease present several striking features in common. They are both examples of hypopituitarism, Case I, with deficient hypophysis secretion from the beginning; Case II, with an earlier stage of hyperpituitarism (acromegaly) succeeded by a period of hypopituitarism following the destruction of the primary hypophysis adenoma (struma). Although, as Cushing has strongly emphasized, cases of hyperpituitarism sooner or later pass into a second stage of hypopituitarism, it seems reasonable to suppose that the transformation in this case is explainable upon the apparent grounds found. Leaving the hypophyseal features of both cases and the acromegalic symptoms of Case II out of the question, the general picture in both cases is the same: a marked obesity or liposis involving all organs, particularly the panniculi, liver and adrenals, with scattered lipoid cells elsewhere. The atypical character of this fat was shown best in the liver and adrenals, and it was most thoroughly studied in the former organ.

LIVER CHANGES IN CASES I AND II.—Both livers show identical changes, perhaps more marked in Case I, although portions of the liver in Case II show as marked changes as any in the liver of Case I. These changes will now be described in detail.

Hematoxylin and Eosin Preparations.—Sections from blocks imbedded in paraffin and stained with hematoxylin and eosin give on first glance the picture of a fatty liver in which the processes of fatty infiltration and fatty degeneration are combined. The lobules are smaller than normal. The cells of the central liver-

zone show numerous fine droplets or vaculations, giving the cell the characteristic "ground-glass" appearance. Through the intermediate and peripheral zones large fat droplets appear, not regularly as in an ordinary fatty liver, but arranged in more or less well defined groups. They are, however, most numerous toward the outer portion of the intermediate zone, and not at the periphery of the lobule. Groups of such large vacuoles occur also in the central zone. Small fat droplets are found also in all of the liver cells except in certain peculiar zones or bands that run around the lobule in the inner portion of the peripheral zone. In these areas there are few fat droplets, the liver cells are small, granular, necrotic or replaced by a cellular fibroblastic area in which remains of liver cells can be made out. In a few lobules these fibroblastic zones touch the periportal islands, but

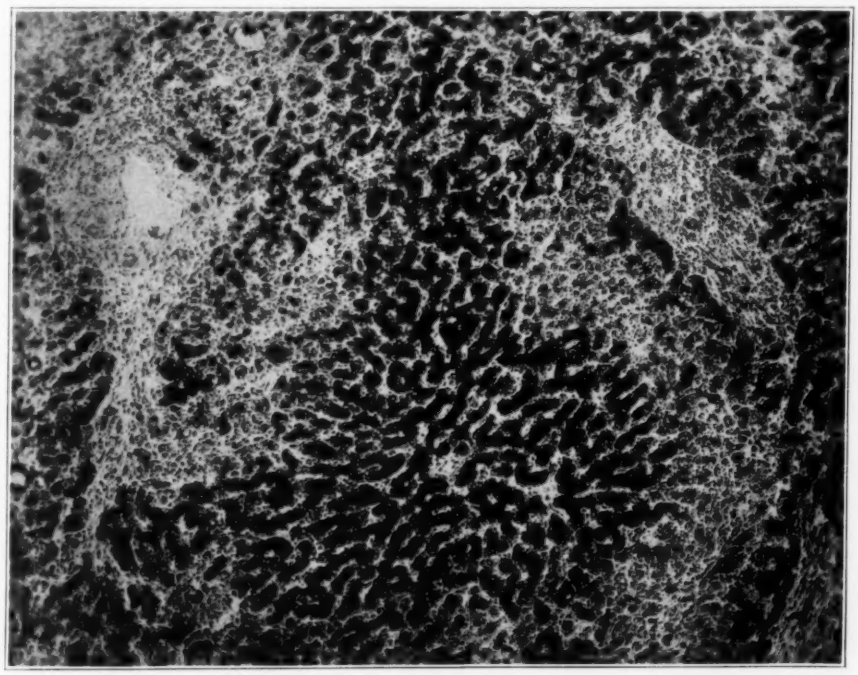

Fig. 1.—Cross-section of liver lobule from liver of Case I. Frozen section, Sudan III and hemalum stain. Central zone and portion of mid-zone and narrow border of peripheral zone show liver-cells filled with minute anisotropic droplets staining deep brownish red, with larger scattered isotropic droplets staining deep yellow-red. Inner portion of peripheral and outer portion of mid-zone show peculiar necrosis with fibroblastic proliferation (intraperipheral necrosis and beginning cirrhosis).

usually a narrow zone of liver cells filled with fine fat granules separates this zone from the trabeculæ. This picture seems to me to be quite unique. I have never seen anything like it in any other liver but in these two cases of hypopituitarism. The periportal tissue is cellular and fibroblastic, frequently showing newly formed bile ducts in numbers. The appearances are those of an early stage of a peculiar form of cirrhosis. It shows more distinctly in the H. and E. preparations of Case I than of Case II; but the peculiarity of this change is best revealed in the sections stained with Sudan III and Scharlach R. and counterstained liver with hemalum. The protoplasm of the outermost cords of cells stains most heavily and has the smallest number of fat droplets. The veins are dilated, but the intralobular

capillaries only moderately so. There is no picture of nutmeg liver or central necrosis, the liver cells around the central veins staining as well as those in the periphery as far as the nuclei are concerned. There is no necrosis, and no pigmentation of the liver cells of the central zone. Iron tests were negative. No pigmented stellate cells were found. Bile-ducts showed no changes.

MICROCHEMICAL STUDY.

Osmic Acid.—In frozen sections treated with one per cent osmic acid, or in sections so treated after formol-fixation the fat content of the liver cells was more decidedly revealed. The small droplets in the central zone of the lobule stained a lighter gray than the large fat droplets scattered irregularly. Some of the droplets are not acted upon at all by the osmic acid or show only the slightest gray tint

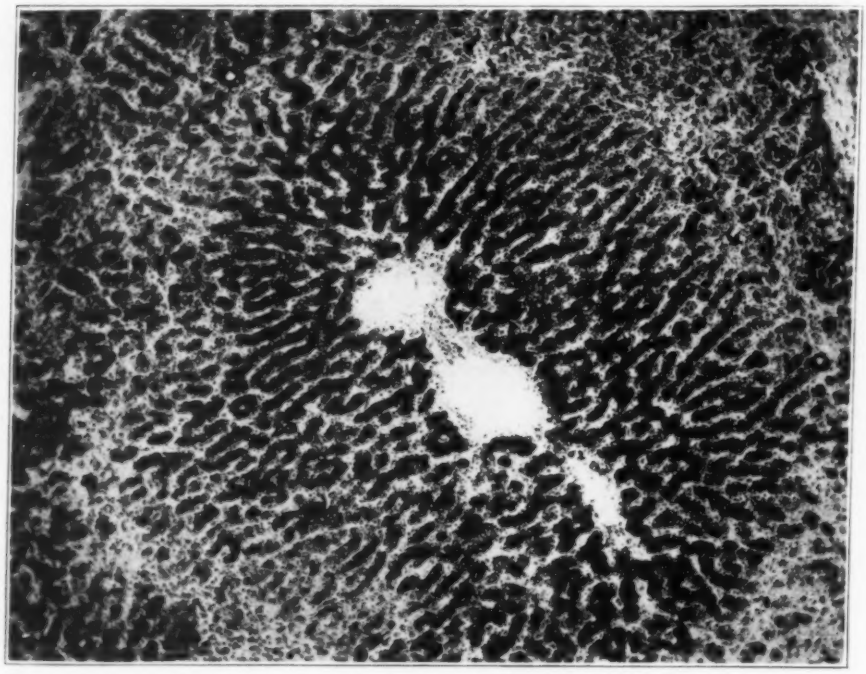

Fig. 2.—Cross-section of liver-lobule from liver of Case II. Frozen section, stained in Sudan III and hemalum. Central zone, greater portion of mid-zone and narrow outer portion of peripheral zone show liver-cells filled with fine anisotropic droplets staining deep brownish red, with scattered larger isotropic droplets staining deep yellow-red. Inner portion of peripheral zone and outer portion of mid-zone show the peculiar necrosis and early cirrhosis (intraperipheral necrosis).

(cholesterol). The peculiar distribution of the fat in the two livers stands out very prominently in the osmic acid preparations. There appear in addition droplets of fat apparently in the lumina of the liver capillaries, and these were mistaken at first for free fat emboli in Case I. They are undoubtedly stellate endothelial cells showing a marked lipoidosis. These give a more gray color-reaction with the osmic. Osmic preparations of adrenals and aorta showed similar color reactions, a large part of the small droplets staining light gray. Sections of the panniculus stained deep brown black.

Sudan III.—Beautiful preparations were obtained with frozen sections

stained in an acetone solution of Sudan III and counter-stained with hemalum. The fine fat droplets in the central zone and throughout the liver-lobule stained a peculiar brownish-red, while the majority of the larger droplets stained an orange-red to brick red as does neutral fat (glycerin-esters). Other large droplets were light yellow or pale buff in color. Counterstaining with hemalum brought out the unique distribution of the fat in the liver-lobules. The fat-free areas or zones stand out in these preparations so strikingly and the cellular infiltration and proliferation is so prominently brought out that it is difficult to believe that one is looking at the same tissues seen in the paraffin-hematoxylin-eosin preparations. Occasional fat-droplets are found in these necrotic zones, and such appear to be chiefly in the stellate cells, although a few liver-cells con-

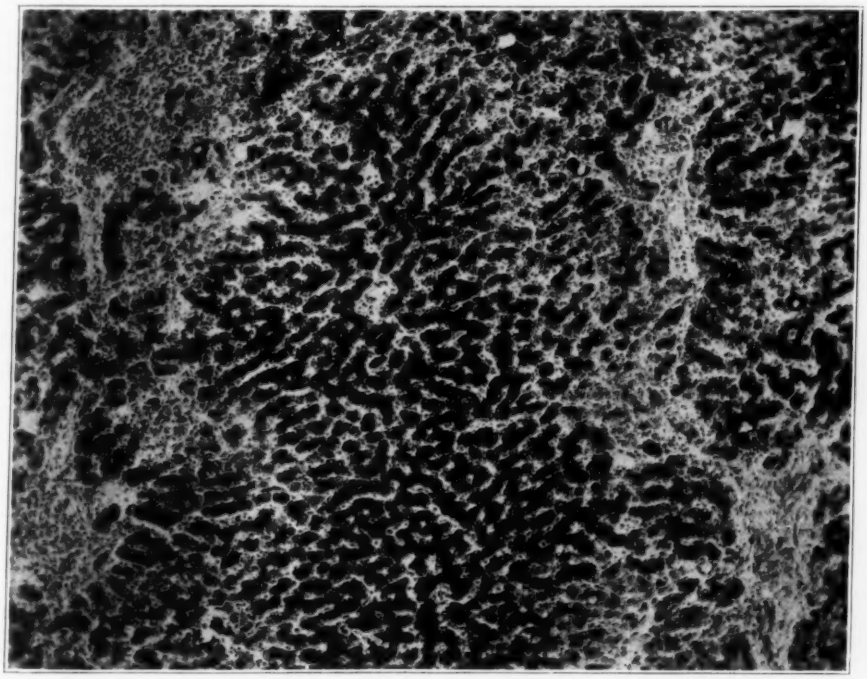

Fig. 3.—Tangential section of liver-lobules from liver of Case I. Frozen section, Scharlach R and hemalum stain. The small black granules in the liver-cells are brownish-red anisotropic fat-droplets (cholesterol-esters), the larger black droplets irregularly scattered are deep red isotropic fat-globules (glycerol-esters). The intraperipheral zone of necrosis and beginning proliferation is well shown.

taining large droplets occur also. The borders of these blue zones, as they appear in the hemalum preparations show a transition from liver-cells containing brownish red granules to cells containing fine brown granules. These brown granules are finer than the red ones and are scattered in the same manner through the cell. They do not give a hemosiderin reaction. Some liver-cells with red granules in one end and brown ones in the other are seen in the borders of these relatively fat-free zones. It is evident, however, that the lack of fat in these narrow zones is due chiefly to a necrosis or disappearance of the liver-cells in these areas, and to a replacement of these by a fibroblastic proliferation.

Scharlach R.—Similar results are obtained in frozen sections stained with an acetone solution of Scharlach R and counterstained with hemalum. The fine granules stain a deep red that has a distinct brownish cast, but their staining is not so intense as that of the large droplets which stain deep bright scarlet red without the brownish cast. The differential staining is about equal in the case of Sudan III and Scharlach R. I have noticed that students vary greatly in their ability to distinguish these differences with these two stains, some seeing it much better in the case of one, others with the other stain. Such results are probably ascribed to differences in color-perception due to individual training or experience in colors. With the Sudan III staining of frozen sections there seems to be at least ten times as much fat in the sections as in the case of osmic acid

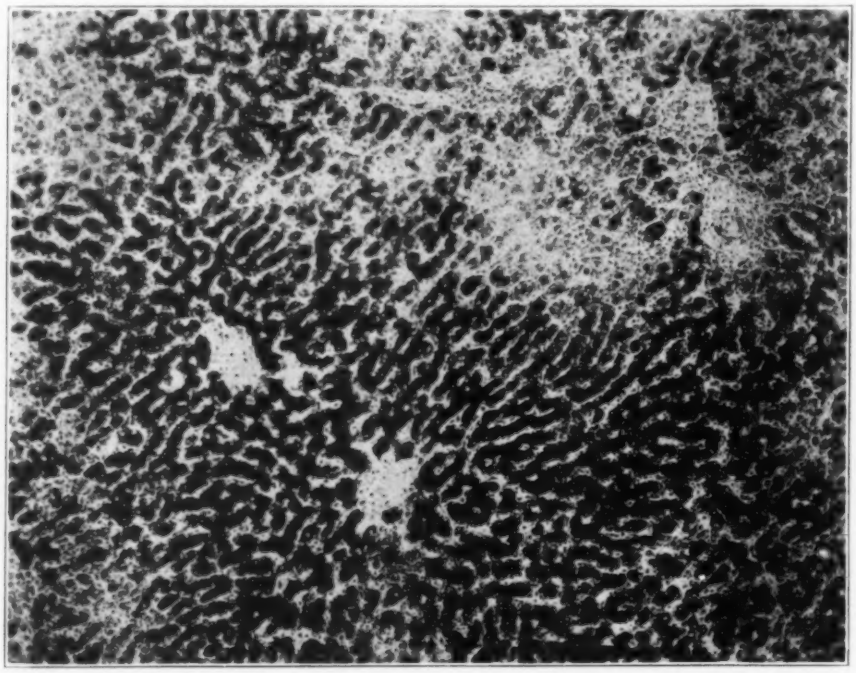

Fig. 4.—Tangential section of liver-lobules from liver of Case II. Frozen section, Scharlach R and hemalum stain. Same appearances as in Fig. 3.

staining. With Scharlach R the amount of fat seems still greater, but this is probably due to the greater intensity of color in the Scharlach R stain.

Nile-blue Sulphate.—With Nile-blue sulphate the fresh frozen sections showed a great variety of color reactions with the fat droplets. The majority of the larger droplets stained red, some pink, some pink-violet, some purple, others deep blue. The majority of the fine droplets stained pink or lavender. The fine droplets in the adrenal cortex and in the lipoid cells elsewhere stain pink or lavender to violet or blue.

Weigert's Hematoxylin.—Deep blue to blue-gray droplets are shown in the larger fat cells. When counterstained with van Gieson's the blue droplets appear as darker brown droplets in the ochre-colored protoplasm.

Indophenol.—In sections stained with a saturated solution of indophenol

in 70 per cent alcohol and contrasted with lithiumcarmin good fat preparations were obtained without any differential staining.

Benda's Method.—This gave no differential staining. Stains for fatty acids and soaps negative. Other staining methods (Smith-Dietrich's, etc.) were negative as far as differential staining was concerned.

Optical Appearances.—With polarized light doubly refractile (anisotropic) droplets were numerous in all the cells containing fine fat droplets. The larger cell droplets or crystals were chiefly isotropic, but anisotropic droplets were present in all cells containing fat. Crystal-like bodies appeared in the droplets on freezing. Warming caused the doubly refractile droplets to disappear.

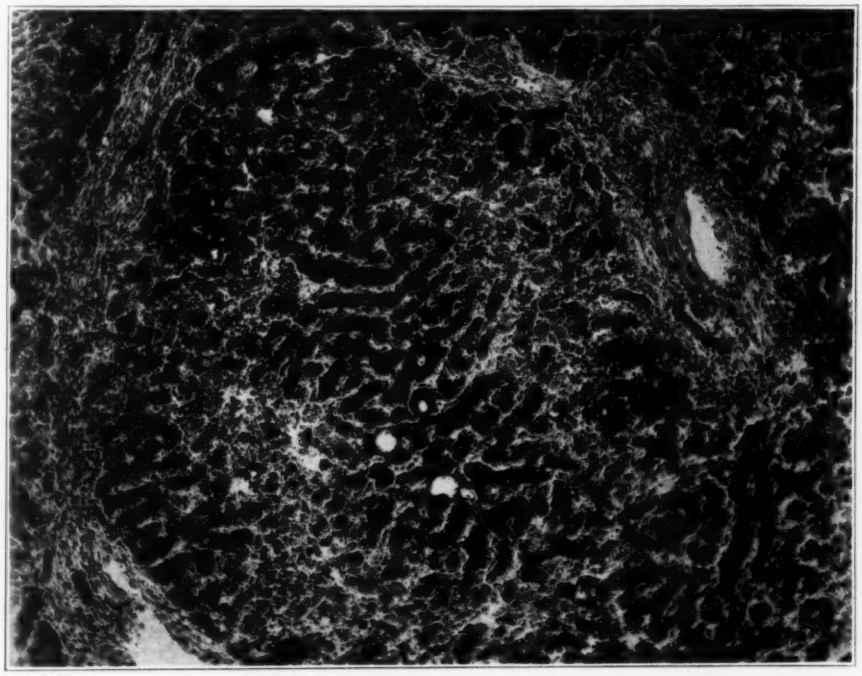

Fig. 5.—Section of liver-lobule from liver of Case I. Frozen section, Scharlach R and hemalum. Slightly higher magnification. Shows same character of lipin changes as in preceding figures, but in addition a more marked degree of peripheral necrosis and proliferation (more advanced cirrhosis).

Alcohol, ether, xylol and hot paraffin dissolved all of the droplets from the cells leaving vacuoles. Acids and alkalies did not dissolve them. Chromate fixation caused the larger cell droplets to become brown or greenish. No effect was noted upon the finer ones. Formol fixation had a marked effect upon the largest fat droplets in the panniculi, causing a formation of yellowish or green needle-shaped crystals in the cell. Portions of the two livers have been kept in formol and given each year to laboratory classes for fat study. While the differential reactions shown by Scharlach R, Sudan III and osmic acid are still obtainable they are not as sharp as at first; and the Nile-blue sulphate reactions have become very capricious. At present many droplets stain blue or purplish blue. Long-continued residence in formol undoubtedly affects the fats of the

cells. The most accurate conclusions therefore would be those drawn from the study of perfectly fresh tissues.

From the fat-reactions shown above it is evident that the livers of these two cases of hypopituitarism present an unusual lipin content. The presence of numerous anisotropic droplets, staining a lighter gray with osmic acid, brownish or yellowish red with Sudan III and Scharlach R, and pale pink with Nile-blue sulphate make it very probable that these are cholesterol-esters. Other lipoids may be mixed with these and with the glycerin-esters in these droplets. The lipin combination may be very complex. It is quite evident that our microchemical tests at the present time are not sufficiently reliable to differentiate accurately the different lipoids. We were unable to make satisfactory chemical studies of these lipins

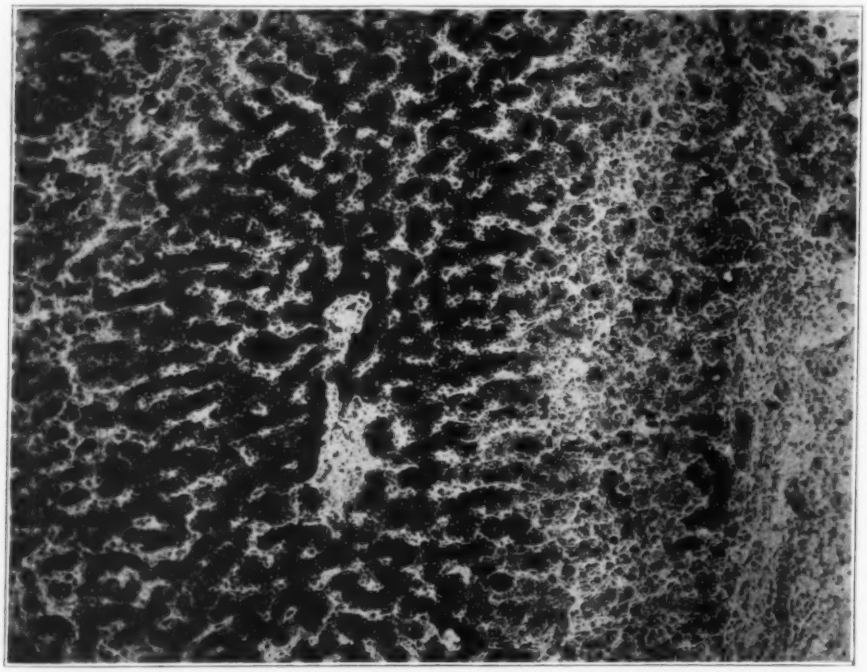

Fig. 6.—Section of liver-lobule from liver of Case II. Frozen section, Scharlach R and hemalum. Slightly higher magnification. Shows the same lipin changes, and the marked peripheral necrosis and beginning cirrhosis.

because of the fact that the greater part of the material was distributed through various fixing fluids at the time of the autopsy. The reactions are sufficiently characteristic, however, to enable us to state that the fatty changes in these cases are not those of ordinary fatty infiltration and degeneration but represent a mixed glycerin-ester and cholesterol-ester lipoidosis. Whether lecithin is present or not it is impossible to say. It is also evident that many of the liver cells contain chiefly cholesterol-esters. Further, this cholesterol lipoidosis is not confined to the liver, but occurs to a marked degree in the adrenals, in the panniculi, and to a lesser degree in scattered cells or groups of cells in other organs and tissues (spleen, aorta, kidney, etc.). In the panniculi the bulk of the lipins are probably glycerin-esters. The condition is then one of abnormal lipin metabolism

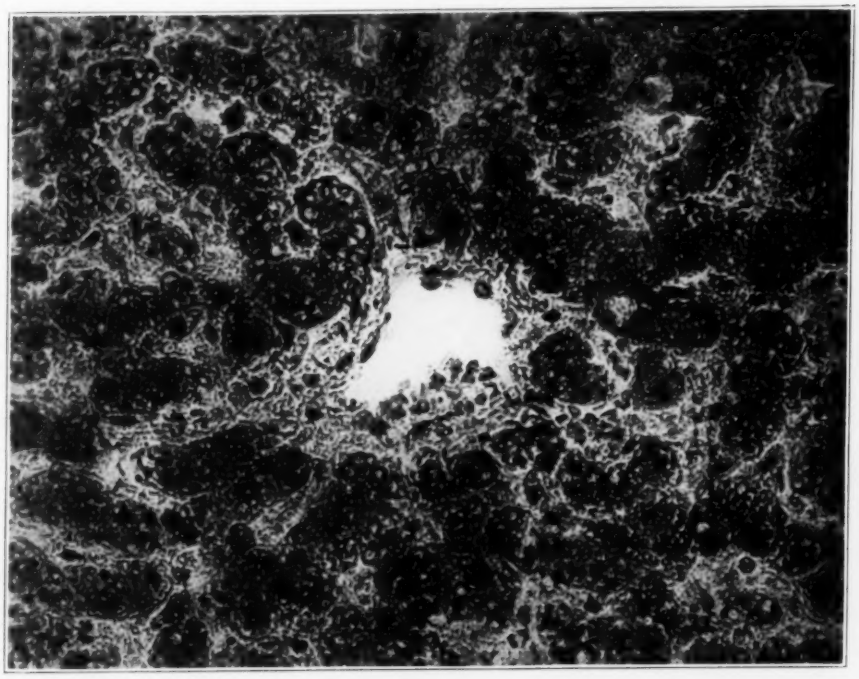

Fig. 7.—Central zone of liver-lobule from Case I. Frozen section, Scharlach R and hemalum. High power. Cells packed with small anisotropic brownish-red droplets and occasional larger isotropic deep-red globules. Cholesterol- and glycerol-ester steatosis.

Fig. 8.—Border between mid-zone and peripheral zone of liver-lobule from liver of Case I. Frozen section, Scharlach R and hemalum. High power. To the right the zone of beginning necrosis; to the left the liver cells are filled with minute anisotropic droplets. The larger drops in the center of field with fat partly torn out in freezing are isotropic fat-globules staining deep red.

characterized particularly by cholesterin infiltration or retention (cholesterol steatosis). It is to be compared then to the cholesterol infiltrations obtained by the overfeeding with cholesterol, to the lipoidosis of diabetic lipoidemia and that of Gaucher's disease. It is, however, quite distinct in its histological picture from these conditions, although the differences as far as the deposit of the lipoids are concerned may be more of degree than of kind.

Just what relation the peculiar zonal necroses in the liver bear to the lipin metabolism, it is impossible to say. The peculiar position of this zone, intraperipheral, makes this change a unique feature in the pathology of hypopituitarism. The cause of death of the liver cells is not evident, there are no thromboses of the liver capillaries at this point, no congestion, no cause of local asphyxia, etc. That the necrosis is not altogether recent is shown by the fibroblastic proliferation and the cellular infiltration. It is, in fact, an early cirrhosis beginning, not at the periphery of the lobule, but a little distance within it, leaving outside of it a narrow zone of unaffected liver cells. In the necrotic zone all stages of atrophy and necrosis of the liver-cells can be seen. At the borders the cytoplasm of the liver cell is filled with fat-granules; these change to brownish granules, which, in turn, as the cells become smaller, become less and less visible, until only pale colorless cell bodies remain. The best explanation of this peculiar zonal necrosis is on the assumption of some toxic substance brought in the blood stream and acting upon the liver cells at this point; or else, as a result of metabolic disturbances confined to this zone of cells.

I have searched in vain through the literature for observations of a similar intraperipheral zonal necrosis, but have found nothing comparable to this form. The mid-zonal necroses associated with marked fatty infiltration of the central zone with hyperemia and necrosis of the intermediate zone are not like this; nor does it resemble the mid-zonal necrosis seen in yellow fever. There is no hyperemia, the capillaries toward the central vein are not obstructed, and the cells of the central zone, while filled with fat droplets, are not larger than normal liver cells. Finally, the necrosis is more peripheral than mid-zonal, involving the inner portion of the peripheral zone.

Both cases showed a very striking postmortem increase in temperature. Thermometer readings were taken of the second case. Seven hours after death, with all of the internal organs removed, lying on a metal table over which cold water was running, the panniculus in the flaps of the abdominal wall still were unpleasantly warm to the touch, and showed a temperature or 104°. A large slice of the abdominal wall showed the same temperature some time after removal from the body. A clinical thermometer plunged into the liver gave a reading of 108°. It is most probable that this excessive heat-production is related to the abnormal fat deposits, and to postmortem changes in the lipins of the cell.

SUMMARY.

In hypopituitarism there is a peculiar obesity due to infiltration of various cells of the body with a mixture of lipins:—glycerin-esters and cholesterol-esters. This condition of cholesterol liposis is especially marked in the liver and adrenals, but is scattered all over the body. With deficiency of hypophysis

function there appears to be associated a cholesterol retention or infiltration. Hypopituitarism must, therefore, be classed among the xanthelasmic conditions, and is related in kind to diabetic liposis and Gaucher's disease.

The hypophysis is either directly or indirectly concerned with lipin metabolism, particularly with cholesterol steatosis. Postmortem hyperpyrexia may be associated with hypopituitarism and the obesity resulting from the latter condition.

In the livers of two cases of hypopituitarism there occurred a peculiar intraperipheral zonal necrosis, unlike all previously described forms of zonal liver necrosis. Associated with this necrosis is a reparative fibroblastic proliferation, giving rise to the picture of an early intralobular cirrhosis. The relationship of this hepatic change to the hypophysis conditions remains to be shown.

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THE EMPLOYMENT OF CLOSED ETHER ANESTHESIA FOR ORDINARY LABORATORY EXPERIMENTS*

By D. E. Jackson, Ph.D., M.D., St. Louis.

THE maintenance of a regular and reliable ether anesthesia in experimental animals is a matter of much concern to all investigators who find it necessary to carry out this form of work. For student experiments also the needs in this direction are obvious. A simple and cheap method for this purpose, requiring only such apparatus as can readily be secured in practically all laboratories for experimental pharmacology, surgery, or physiology is described in the following paragraphs.

Fig. 1 shows the arrangement of the apparatus complete as it is adjusted to an animal in which a cannula has been inserted into the trachea. The large pan should be about nine and one-half inches in diameter for animals (cats, rabbits) ranging in size up to dogs weighing about 15 to 20 pounds. The depth of the pan should be about three inches. For this purpose I have used a large, round, tinned iron (not aluminum) cake-pan purchased at a ten cent store. For large dogs the pan should be somewhat larger than the one here described. On pans of this type the upper edge is turned outward thus forming a kind of flange with a rounded edge. At a distance of about one-half inch below this a second ring (or flange) must be soldered around the pan. This ring should extend out from the wall of the pan about one-fourth of an inch. The ring may be made of a curved strip of tinned iron, or brass or copper, or a one-fourth inch soft copper tube may be bent around the pan and soldered on. (The copper tube will cost about 45 cents.)

Fig. 2 shows a plan of the pan as seen from above. Into the left hand side of the pan a brass spout made of one and one-eighth inch brass tubing is soldered. This tube enters the pan about half way down its side and extends upward as it passes outward from the pan. At a distance of about two inches from the wall of the pan this tube has an obtuse bend which allows the end of the spout to pass outward in a horizontal direction. This greatly facilitates its attachment to a tracheal cannula or to an air-tight muzzle (Figs. 3 and 4), if the windpipe is not opened. It is important for this tube to be *short* and of comparatively large bore, as this produces the least possible obstruction to the breathing of the animal. A large perforated cork is placed in the horizontal portion of the spout and the side tube of the tracheal cannula is passed tightly through the cork. The free end of the tracheal cannula carries a short piece of rubber tubing which is closed off as the cannula is inserted into the trachea and then attached to the spout of the pan. If a muzzle is used, one of the projecting flanges either at the end or on the side of the muzzle (depending on the position of the animal) is slipped over the horizontal part of the spout on the pan and the connection is made air tight by means of a broad rubber band covering the joint where the tubes overlap. The other flange on the muzzle is then closed with a stopper.

*From the Department of Pharmacology of Washington University Medical School.

From the right-hand side of the pan a brass (oxygen inlet) tube one-fourth inch in diameter passes into the centre of the large pan. At this point

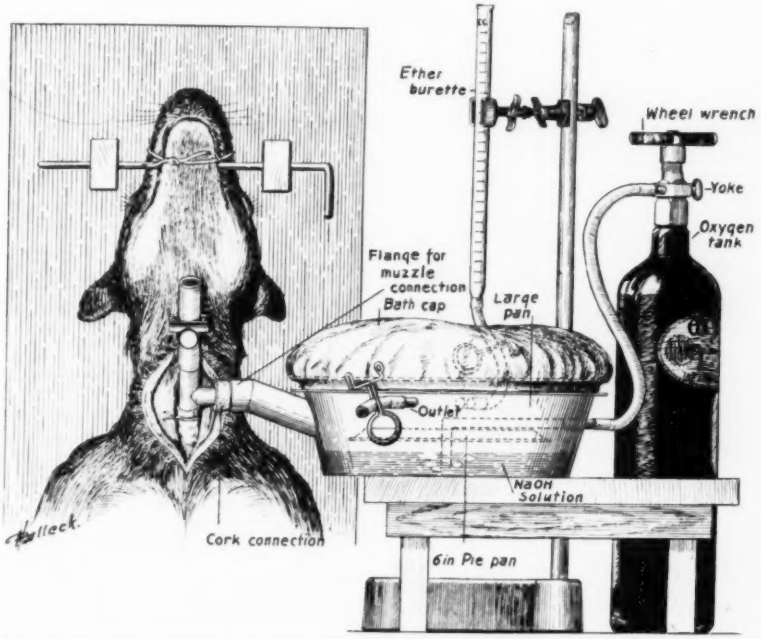

Fig. 1.—A diagram showing the general appearance and arrangement of the apparatus as attached to the side tube of a tracheal cannula. Oxygen is admitted slowly from the tank. The large pan is placed on a small table close to the operating board. The dog and the operating board are shown diagrammatically as if placed in an upright position.

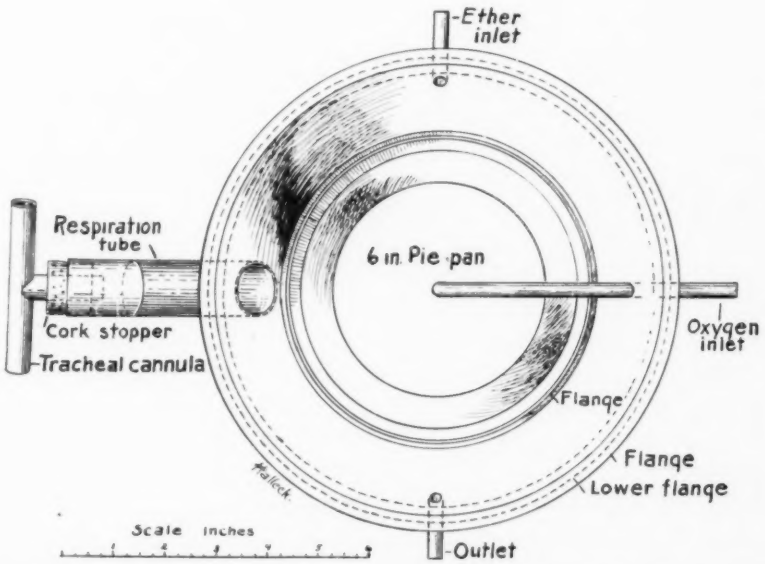

Fig. 2.—A general plan of the apparatus (without the bath cap) as seen from above.

the tube bends downward at a right angle and ends about one-fourth inch from the bottom of the large pan. This tube carries a small (six inch) pie pan (two

can be bought for 5 cents at a ten cent store) which is attached, bottom upwards, just below the angle in the brass tube. The perpendicular portion of the tube passes through a hole made in the centre of the bottom of the small pan. The outer flange of the small pan should be placed at a distance of about one and one-eighth inches above the bottom of the large pan.

Two other brass tubes one-fourth inch in diameter and one inch long are soldered into the upper part of the opposite sides of the large pan just below the lower flange (or ring). One of these tubes serves as an inlet for ether which is admitted from a burette through a rubber tube, while the other brass tube is used as an outlet and carries a short piece of rubber tubing closed by a pinch cock.

When in use a very thin rubber bath cap is stretched over the large pan in such a manner as to allow the rim of the cap to close in between the upper edge (flange) of the pan and the lower flange (ring). This connection must be air tight, but this is practically always accomplished at the first trial if wrinkles are kept out of the cap band. There are many varieties of these caps on the market. The one I have found most satisfactory is made of two pieces of rubber, a round sheet about ten and one-half inches in diameter which serves as the top of the cap, and a second round sheet of approximately the same diameter but having a circular opening about four and one-half inches in diameter cut out of its centre. This opening is the portion which is stretched out to fit over the pan. These two sheets of rubber are cemented together at the outer edges and no puckering or wrinkling of the edges of the cap occurs when the rim is fitted down over the pan. These caps are made of very thin yellow rubber and cost 25 cents apiece (at a large department store). Higher priced caps are to be avoided as they are likely to be made of heavier material, and often have folded edges (which cause leaks of air around the rim of the pan). If reasonable care is taken of one of these caps it will last for many weeks. It should be washed and dried and preferably dusted with powdered starch each time after use.

For use the pan has a layer of strong (not quite saturated) sodium hydrate solution (calcium hydrate may be added if desired) about three-fourths of an inch in depth poured into the bottom. The oxygen inlet tube extends below this solution and when oxygen is admitted from the tank the solution is splashed upward against the small pan. This serves to help the sodium hydrate to absorb the carbon dioxid exhaled by the animal. The small pan prevents the solution from splashing up into the spout and reaching the lungs of the animal. Ether is admitted from a burette and only very small quantities are needed. Injections from the burette may often not exceed one-half or one cubic centimeter for half an hour after the animal is once thoroughly well anesthetized. Some ether usually has to be added from time to time, but the greatest danger in the use of the apparatus is the giving of too much ether. This is well shown in Fig. 5, in which one cubic centimeter of ether was injected when the animal was already fairly deeply anesthetized. This ether was later allowed to escape (by opening the screw clamp at the end of the tracheal cannula).

If a muzzle similar to the one shown in Figs. 3 and 4 is used the animal may be attached to the operating board and anesthetized with ether injected

into the pan. I have, however, usually anesthetized the animal on the floor and then inserted a tracheal cannula which is at once attached to the pan. This method is more rapid but more ether may be wasted in starting the anesthesia.

Regarding the cost of anesthesia for animals by this method I may give the following data which is only an approximation of the expense which varies greatly with the size (species) of the animal and with the nature of the experiment. In a series of ten experiments on dogs (some very large, others

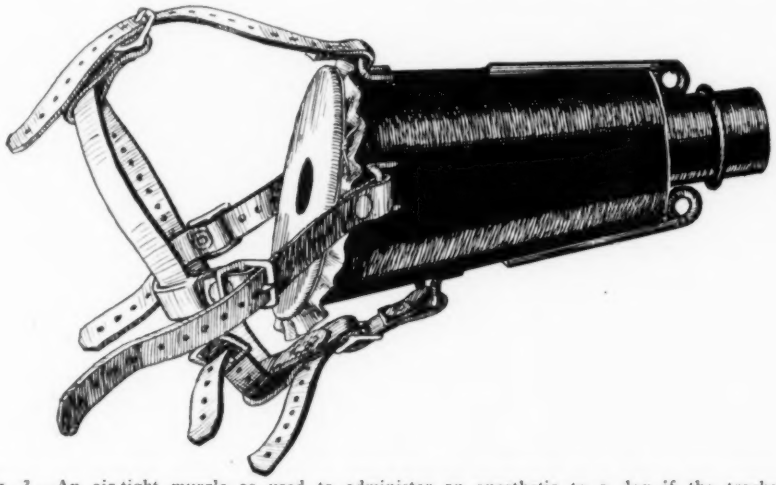

Fig. 3.—An air-tight muzzle as used to administer an anesthetic to a dog if the trachea is not opened. The rear end of the metal (brass) cylinder is covered with a disc of heavy rubber dam which is perforated with a three-fourths inch hole near the center. The dog's nose and mouth are passed into the muzzle through this hole.

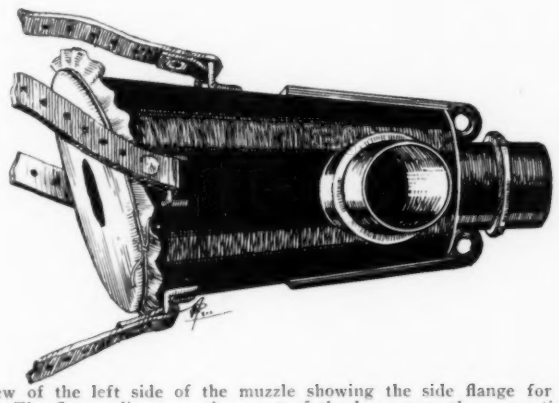

Fig. 4.—A view of the left side of the muzzle showing the side flange for use when the animal is lying on its back. The flange slips over the spout of the large pan, the connection being covered with a broad rubber band.

small) in which the anesthesia lasted from perhaps one hour up to seven hours and ten minutes I used one forty gallon tank full of oxygen, approximately two pounds of sodium hydrate and about one pound of ether. The oxygen (in 40 gallon tanks, one of the most expensive sizes) cost $2.25, the sodium hydrate cost $0.50 per pound (abnormally expensive) and the ether $0.36 per pound. This indicates that the cost of these experiments was about $0.36 per animal, and as the average duration of each experiment was approximately four hours

the average cost per hour was about nine or ten cents. This must necessarily vary greatly, however, with the size of the animal, for a small dog will use very much less oxygen and sodium hydrate than a large one. The cost of the

Fig. 5.—Blood pressure (upper) and respiration (lower) tracings from a dog. The animal was fairly deeply anesthetized and had received no ether for some time. At the point marked by the first two crosses 1 cubic centimeter of ether was injected into the apparatus from the burette. The effect of this comes on gradually and progressively until at the second set of crosses the tracheal cannula was opened to the outside and the animal allowed to exhale the excess ether. Recovery then rapidly occurred.

ether is very small in any case. The chief point to be considered in this method is, however, not the initial cost of the chemicals, but the greatly improved and reliable form of anesthesia. For in a closed method such as this, one "dose" of ether is retained with but exceedingly small variations within the animal over prolonged periods of time, and the regularity of the anesthesia compares

more with that produced by a "dose" of morphine than with the ordinary form of anesthesia carried on by the open methods, with ether bottles, etc. One has full command of the depth of the anesthesia and can vary it at any time. Chloroform, ethylchlorid or nitrous oxid may also be used by this method but for ordinary experimental work ether is preferable.

There were three points about which I was in doubt before I had carefully tried out this method. These were first, the question as to whether or not the movement of the respired air (or vapors) above the sodium hydrate solution would be sufficient to cause a satisfactory absorption of the carbon dioxide exhaled by the animal. With very large dogs I believe that CO_2 may thus accumulate to some extent at times especially if the alkali solution is not sufficiently strong. With smaller animals (also rabbits, etc.) the CO_2 causes no disturbance and is apparently practically completely absorbed as soon as exhaled from the lungs. With a larger form of apparatus this absorption would undoubtedly be sufficient for any animal. It was with this object in view that the wide shallow cake pan was used so as to provide for a large absorbing surface.

The second point about which I was in doubt concerned the possible accumulation of too much moisture in the air breathed. It is to be remembered in this connection that dogs give off exceedingly little moisture in the form of sweat and excretion of water by the lungs becomes of especial importance. This accumulation of watery vapor, however, depends mainly on temperature. This was the third point about which I was especially concerned. In practice it does not appear, however, that either of these factors causes any special disturbance. This is probably due to the use of a considerable volume of cold alkali solution and the further rapid cooling of the air to room temperature which can occur through the very thin and flexible bath cap. A further factor in the cooling also is the admission of oxygen from the high pressure tank. The sudden expansion of this gas as it bubbles through the hydrate solution cools very considerably the oxygen admitted. Unless the dog be too large for the capacity of the apparatus none of these factors causes any objectionable features in the anesthesia.

It is important for the alkali solution to be cold, however, when it is used. The alkali should be dissolved and the solution allowed to cool for several hours before it is employed for an experiment. In one instance, by mistake, some sticks of sodium hydrate were added to the solution (which had been used before) in order to strengthen it just before an experiment was started. These fresh sticks in dissolving raised the temperature of the alkali solution in the large pan considerably. Correspondingly it was found that the rectal temperature of the animal rose to 105 degrees Fahrenheit. In average experiments, however, the temperature usually remains practically constant at the point registered when the apparatus is attached to the animal, but in long experiments, or following severe operations, the temperature slowly falls. It has seemed to me that the temperature often thus remains up to normal thereby conserving the vitality of the animal at times when ordinary open methods of anesthesia would considerably weaken the animal through heat loss.

Certain interesting features are often brought out by this method of anes-

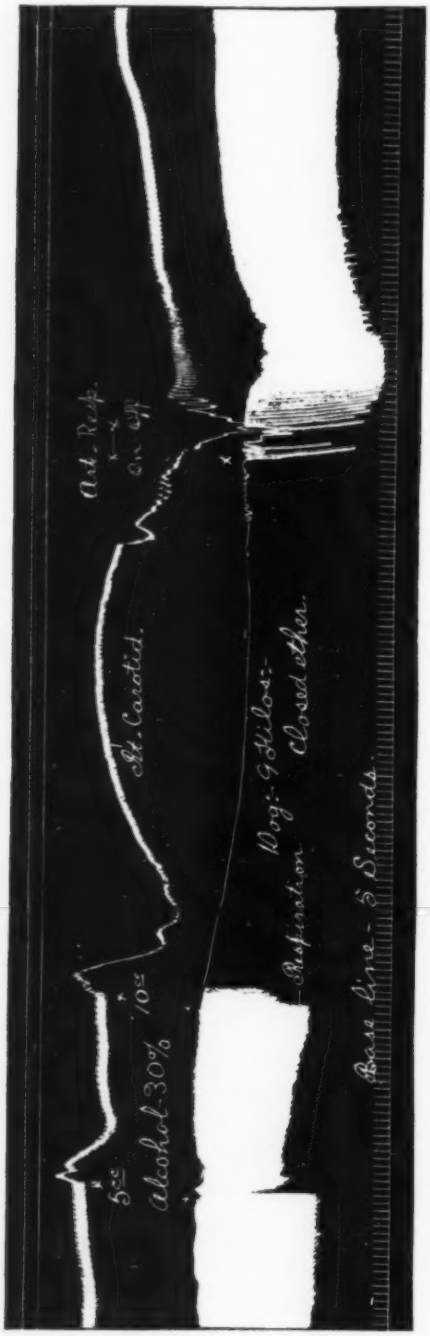

Fig. 6.—Tracings of blood pressure and respiration from a dog. Injection of ethyl alcohol as indicated stopped the respiration but the heart and circulation were very much less affected probably because of the abundant supply of almost pure oxygen.

thesia. One of these is illustrated in Fig. 6 which shows the action of alcohol on the circulation and respiration. The second injection (intravenously) of ten cubic centimeters of 30% alcohol completely stopped the respiration. There was a considerable initial fall of blood pressure followed by a rise

(asphyxial?), but the pressure did not again soon begin to fall rapidly as would ordinarily occur. On the contrary the pressure remained high for a very considerable period of time during which the respiratory movements were entirely absent. Finally, as the blood pressure started to fall rapidly, the lungs were inflated a few times artificially and then the animal at once started rapidly to

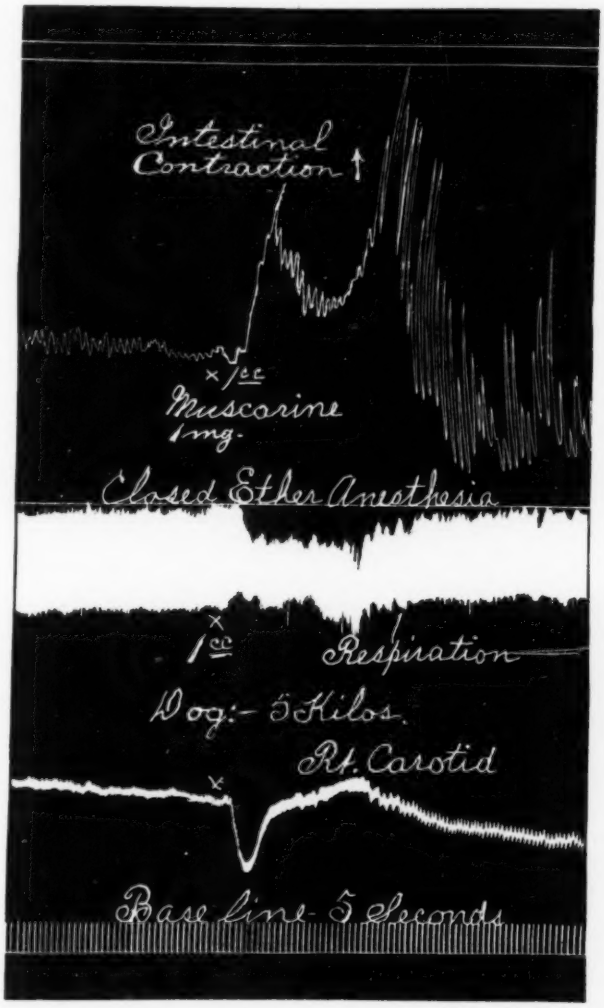

Fig. 7.—Intestinal contractions, respiration and blood pressure of a dog under closed ether anesthesia. Injection of 1 milligram of muscarine caused marked reactions as indicated in the tracings.

recover. The point of interest here is the very long period during which the animal lived in very good condition while the respiration was stopped. It would appear that the approximately pure oxygen in the apparatus, with which the lungs were in fairly direct communication through the trachea, must have been in part responsible for the result observed.

Another point of interest is the fact that, as a rule, one must from time to time add small amounts of ether to the apparatus to maintain the anesthesia

at a constant depth. It would seem that this may be due to the very gradual destruction of small quantities of the drug or to its excretion in traces from the tissues of the animal. I have suspected that a very limited amount may thus escape through the mucous membranes of the mouth. Other small quantities may be excreted into the urine or pass into the intestinal contents or into the structure of the bones, etc., when the depression of the central nervous system might be decreased a little thus allowing slight symptoms of recovery to appear. It is also possible that small traces of the drug may leak out through the rubber bath cap. In all cases, however, it is strikingly evident that only the smallest amounts of ether can either be oxidized or excreted during the course of the longest anesthesia. When the breath is allowed to escape into the open air the ether is of course rapidly excreted by the lungs.

DIAGNOSIS OF CANCER*

By Max Kahn, M.D., Ph.D., Pittsburgh, Pa.

THE early diagnosis of cancer is so important for the welfare of the patient, that clinical methods alone are only of limited and usually precarious significance. The laboratory has been striving for a number of years to develop a test for cancer which should be as reliable as the Wassermann test is for syphilis.

In the past two decades or so, quite a number of methods have been suggested for the early diagnosis of malignant neoplasms. Not one of the methods thus far recommended has proved specific and infallible. The enthusiastic claims of the originator of each laboratory procedure could not be corroborated by the various other workers in this field. Nevertheless, a combination of several methods may aid the clinician very much in the early diagnosis of cancer. The tests thus far suggested may be classified as follows:**

A. Microscopical.
   1. Examination of shreds of pieces of tissue.
   2. Examination for bacteria.
   3. Examination for protozoa, etc.

B. Biological.
   1. Hematological.
      a. Analysis for occult blood.
      b. Presence of specific bodies in blood.
      c. Serological.
         i. Deviation of the complement.
         ii. Anaphylactic studies.
         iii. Presence of precipitins, lysins, hemolysins, etc.
         iv. Presence of antitrypsin.
         v. Toxicity of serum.
         vi. The Abderhalden reaction.
         vii. The Meiostagmin reaction.
   2. Biochemical.
      a. Gastric contents' analyses.
         i. The glycyl-tryptophane and other polypeptid reactions.
         ii. The Salomon albumin test.
         iii. Anaphylactic studies.
         iv. Enzyme tests.
      b. Urinary analyses.
         i. Enzyme tests.
         ii. Metabolism studies of nitrogen, sulfur and phosphorus.

C. Clinical methods.
   1. Test diets—Glutzinski, etc.
   2. Skin reactions and vaccine methods.

D. Chemical.
   1. Analyses of gastric contents.
      a. Presence of lactic, acetic, oleic acids, etc.
      b. The hypoacidity determinations.
      c. Tests for blood.
      d. Analysis for nitrogen, albumin, phosphorus, etc.

*From the Biochemical Laboratory, Western Pennsylvania Hospital, Pittsburgh, Pa.
**Kahn, Max: Arch. Diagnosis, July, 1914.

2. Analysis of urine.
   a. Colloidal nitrogen estimation.
   b. Neutral sulfur determination.
   c. Determination of oxyproteic acid.
   d. Sundry other reactions.
3. Examination of puncture fluids from serous cavities.

We have compiled in a table of statistics the findings of various scientists, who have reported either favorable or unfavorable results with the more important of these tests.

TESTS.	FAVORABLE				NON-FAVORABLE			
	CANCER		NON-CANCER		CANCER		NON-CANCER	
	% +	% −	% +	% −	% +	% −	% +	% −
The Abderhalden Reaction	100	0	6	94	30	70	10	90
The Meiostagmin	89	11	0.9	94.1	83	7	40	60
The von Dungern	89.8	10.2	7.2	92.8	40	60	35	65
The Brieger-Trebing	93.6	6.4	30.7	69.3	56	44	75	25
The Salomon Test	95	5	10	90	60	40	10	90
The Salkowski-Kojo Test	93	7	12	88	70	30	20	80
The Salomon-Saxl Test	85	15	7	93	80	20	45	55
The Kelling Test	81	19	18	82	70	30	35	65
Glycyl-Tryptophane	95	5	0	100	95	5	90	10
Oxy-proteic Acid	90	10	8	92	80	20	40	60

We have given special attention to the Salomon test for gastric cancer, and to the Salkowski-Kojo and the Salomon-Saxl test for malignancy of any part of the body.

I. SALOMON TEST FOR GASTRIC CANCER.*

On the assumption that malignant disease of the stomach is accompanied by degeneration processes which liberate debris and protein matter into the stomach cavity, Salomon recommended a test which he thought was diagnostic of cancer of the stomach. This theoretical assumption is in accordance with our knowledge of cancer in general, and the results reported by various observers would seem to bear out the theory. It must be remarked, however, that such diseases as gastric ulcer would also cause a gastroalbumorrhea, as has been demonstrated by several opponents of Salomon's test.

Salomon's method for testing the stomach contents for the albumin fraction is as follows: The stomach is first carefully washed on the evening before testing, after a preliminary nonalbuminous fluid diet had been administered for twenty-four hours. On the next morning the stomach is thoroughly washed with normal saline solution (400 c.c.) the same fluid being repeatedly used and then tested for the quantity of nitrogen by the Kjeldahl method and for the quantity of albumin by the Esbach method.

Salomon found the nitrogen content in noncarcinomatous cases to be between 0 and 16 milligrams per 100 c.c. of fluid. His study of six cases of cancer of the stomach revealed between 10 and 70 milligrams of nitrogen per c.c., and the albumin content was between 0.06 and 0.5 parts per thousand. According to Salomon a case is extremely suspicious of carcinoma if the nitrogen con-

*Kahn, Max, and Jacobowitz, I.: Med. Rec., New York, Dec. 11, 1915.

tent is more than 20 milligrams per 100 c.c. of the fluid, or if the Esbach test gives a distinct precipitate.

Wolff and Junghans modified the Salomon technic somewhat. They determined the albumin by the phosphotungstic acid reagent. They obtained very good results. Smithies found that the Wolff-Junghans modification is of decided value. Another modification of this test has been recommended by Goodman, who desired to eliminate the labor involved in a Kjeldahl determination. He analyzed the gastric contents for phosphorus.

Siegel concurred with Salomon's opinion, concluding from his own results that a figure over 25 milligrams of nitrogen per 100 c.c. is suspicious of gastric cancer, Orlowski, Schittenhelm and Lowes, Zirkelbach, Witte, and Schupfer are convinced that the Salomon test is of value. Gerster regards this test as useful in cancer of the lesser curvature without stenosis, unless the cancer has formed on an old ulcer, in which case the little hydrochloric acid present would digest the albumin present. Zirkelbach, however, is of the opinion that the minimum nitrogen content suggestive of cancer is 30 milligrams nitrogen per 100 c.c. of the washing fluid. Berent and Guttmann, Romano, Minkowski and Yague have reported very unfavorable results with this test. Goodman, on the basis of his findings with his modified technic, concluded that: (1) In normal individuals and in persons suffering from diseases exclusive of carcinoma of the stomach, the Salomon test gives more than 20 milligrams of nitrogen per 100 c.c. of wash water; (2) not all cases of gastric carcinoma reveal more than 20 milligrams of nitrogen—the absence of ulceration is probably responsible for this; (3) the test is by no means pathognomonic and can be considered as contributary only to the other symptoms; (4) the phosphoric acid of the wash water of a noncarcinomatous case is less than 10 milligrams per 100 c.c., whereas in cancerous conditions it usually exceeds 10 milligrams.

Mention should be made of the work of Clarke and Rehfuss, which was communicated by Hawk before the Columbia University Biochemical Association. The following is a summary of their findings: "The protein content of the gastric juice was investigated by the method of Wolff, namely, by successively diluting the gastric juice and adding phosphotungstic reagent. It was found that the normal gastric juice *per se* contained only traces of protein, never giving a reaction in dilutions greater than 1:40. The protein content of the specimens removed by the fractional method, after administration of Ewald meals, was also determined. The macerated Ewald meal *in vitro* never gave a reaction in a dilution greater than 1:40. Treated in the incubator with artificial gastric juice, the authors were unable to demonstrate an increasing content due to the effect of the gastric juice on the proteins of the bread. If, therefore, material removed from the stomach at intervals develops a greater protein content than the theoretical content due to the action of the gastric juice on the bread proteins, we are able to say that it comes from other sources than bread." In cancer the authors found a high protein coefficient.

We have found the Salomon test to be of distinct value in the diagnosis of cancer of the stomach. It will be seen from the accompanying tables that the figures obtained in gastric malignancy were very much higher than the figures

ALBUMIN IN THE GASTRIC WASHINGS OF NONGASTRIC CASES.

NO.	NAME.	DIAGNOSIS.	NITROGEN MGS. IN 100 C.C.	ALBUMIN PARTS P.M.	
				ESBACH.	TSUCHIYA.
1	J. M.	Diabetes	12.5	Traces	Traces
2	I. B.	Diabetes	9.4	None	Traces
3	N. R.	Pulm. Tbc.	7.3	None	None
4	F. M.	Fracture	5.9	None	Traces
5	A. J.	Fracture	1.4	None	None
6	S. H.	Syphilis	8.2	None	None
7	A. K.	Syphilis	5.4	None	None

GASTROALBUMORRHEA IN CARCINOMA OF THE STOMACH.

NO.	NAME.	NITROGEN MGS. IN 100 C.C.	ALBUMIN PARTS P.M.	
			ESBACH.	TSUCHIYA.
8	Mrs. W.	69.8	0.75	0.8
9	M. W.	52.7	0.6	0.75
10	F. C.	71.5	0.65	0.8
11	S. G.	47.9	0.6	0.8
12	E. F.	33.2	0.4	0.5
13	M. G.	44.8	0.5	0.7
14	Z. H.	51.2	0.5	0.6
15	A. T.	32.4	0.4	0.5
16	D. S.	67.4	0.75	0.9
17	E. M.	62.8	0.8	0.9
18	W. J.	32.5	0.6	0.8
19	A. H.	27.9	0.4	0.6
20	G. T.	35.5	0.5	0.75
21	G. F.	29.3	0.4	0.75
22	J. L.	51.2	0.5	0.6
	IN CANCER OF THE ESOPHAGUS.			
23	N. Z.	62.7	1.2	1.5
24	N. H.	28.4	0.4	0.6
25	J. T.	43.9	0.6	0.8

GASTROALBUMORRHEA IN NONCANCEROUS DISEASES OF THE STOMACH.

NO.	NAME.	DIAGNOSIS.	NITROGEN. MGS. IN 100 C.C.	ALBUMIN PARTS P.M.	
				ESBACH.	TSUCHIYA.
26	B.	Gastric ulcer	25.5	0.2	0.2
27	S. K.	Gastric ulcer	32.4	0.4	0.4
28	M. F.	Gastric ulcer	41.2	0.5	0.6
29	M. M.	Gastric ulcer	33.7	0.4	0.3
30	B. W.	Gastric ulcer	35.9	0.4	0.5
31	J. J.	Gastric ulcer	22.6	0.3	0.3
32	R. K.	Gastric ulcer	12.8	None	Trace
33	K.	Cardiospasm	1.5	None	None
34	M.	Acute gastritis	38.9	0.5	0.5
35	F.	Hyperacidity	12.5	None	Traces
36	Katz	Gastrectasis	3.9	None	None
37	L. B.	Tabetic crises	2.4	None	None
38	T.	Hyperacidity	1.7	None	None
39	L. K.	Chronic gastritis	19.8	0.2	0.3
40	J. U.	Anacidity	5.5	None	Trace
41	M. D.	Appendicitis	2.6	None	None
42	S.	Hernia	2.4	None	None
43	M. M.	Intestinal stasis	4.7	None	None

found in other diseases of the stomach. Care must be observed to eliminate gastric ulcer and acute inflammation of the stomach mucosa. A negative Salomon test is significant of a nonmalignant condition. A positive test has to be judged discriminatingly.

We wish to call attention to the fact that ordinary, routine examination of the gastric contents after an Ewald test meal is of but little value as an aid to diagnosis of gastric carcinoma.

Graham and Guthrie analyzed the gastric contents of 150 patients suffering with carcinoma ventriculi. They obtained the following results, which we shall present in the form of a table:

Free hydrochloric acid present in	70	cases
Free hydrochloric acid (no blood, lactic acid, food) in	46	"
Blood present in	80	"
Blood and lactic acid present in	20	"
Blood and food present in	15	"
Blood and food and lactic acid present in	30	"
Food remnants present in	62	"
Lactic acid present in	64	"

II. THE SALKOWSKI-KOJO AND THE SALOMON-SAXL TESTS.*

Salkowski-Kojo Test.—In 1892, Töpfer found that the urine of patients suffering from cancer contained a very large amount of "extractive substance." This "extractive substance" was calculated by first determining the quantity of total nitrogen and then subtracting, from this amount, the sum of the nitrogen values for urea, uric acid, and ammonia, of the same urine. Bondzynski and Gottlieb, five years later, reported that the nitrogen in oxyproteic acid, in the urine, was 2 to 3 per cent of the total urinary nitrogen. Salkowski, and Hess and Saxl, using different procedures, concluded that the oxyproteic acid portion of the alcohol-precipitable substances is increased in the urine of human beings suffering from carcinoma.

Salkowski and Kojo, in a preliminary communication, recently suggested several methods for the determination of colloidal nitrogen in the urine. A year later, Kojo published the results of a comparative study of the various procedures suggested in this connection. Kahn and Rosenbloom studied the zinc-sulfate-precipitable, colloidal, nitrogenous material from the urine of normal subjects, as well as of carcinomatous patients, and concluded that the amount of colloidal nitrogen was invariably increased in carcinoma. They also found that diseases like myocarditis, diabetes, leukemia, and anemia, likewise gave a high colloidal-nitrogen index. They concluded that this quantitative test was not specific for cancer. Kahn and Rosenbloom studied the amount of colloidal nitrogen in the urine of a dog suffering from a malignant neoplasm. In this case they used dialysis as a part of the method and found that the quantity of colloidal nitrogen was much greater in the urine of the diseased dog than the amount present in the urine of normal dogs.

Volpe found that the colloidal-nitrogen index is of special value in cancer diagnosis. Mancini, using the Salkowski method, found that there were in-

*Goodridge, F. G., and Kahn, Max: Biochem. Bull., 1915, iv, 118.

creased eliminations of colloidal nitrogen in the urines of patients afflicted with cancer, but this increase also occurred in pneumonia and pleurisy. Seminov reported that the colloidal nitrogen output is low in normal individuals and is increased in cancer patients. He concluded that although the normal index excludes the possibility of a malignant growth, the increased amount of colloidal nitrogen in the urine is not specific for carcinoma. Konikov found that the average amount of colloidal nitrogen in the urine, as determined by the Salkowski-Kojo method, was 1.68 per cent of the total nitrogen in normal cases, and 2.47 per cent in carcinomatous individuals. Of 73 cases of cancer investigated by him, only 9 showed a higher coefficient than 2.5 per cent.

According to Marcel, Labbe, Dauphin and others, on the other hand, increase in the urinary colloidal nitrogen is an index of a derangement of nitrogenous metabolism; and while it may serve to detect functional insufficiency in the liver, it is not at all specific for cancerous states. Carforio, also, concluded that the colloidal nitrogen index is not pathognomonic of cancer.

Salomon-Saxl Test.—Salomon and Saxl have described a neutral-sulfur reaction in the urine. Like all other tests in this connection, it has given excellent results in some hands but, in others, has proved valueless. The abnormal constituent in the urine of carcinomatous patients is a neutral-sulfur fraction, the sulfur of which can be split off by means of hydrogen peroxide, and can be determined as barium sulfate. Positive urines yield 0.010 to 0.018 gm. of barium sulfate from this fraction, for 100 c.c. of urine. Of 41 carcinoma cases examined by Salomon and Saxl, 30 were positive, 4 faintly positive, 1 questionable, and 6 negative. Of 182 normal urines, 6 were positive, 3 faintly positive, 1 questionable and 172 negative.

Peterson divided his cases into three classes. (A) Clinically noncancerous suspects: of 26 patients examined, 25 gave a negative Salomon and Saxl neutral-sulfur reaction. (B) Clinically cancer suspects: of 20 cases examined, 5 were negative, 2 alternately positive and negative reactions, and 13 cases positive. (C) Manifest cancer: of 19 cases, 17 always gave a good positive reaction; the two negatives were icteric and cachectic. Dozzi found that the test was invariably negative in all his patients free from cancer or tuberculosis, but the frequency of the positive responses in tuberculous patients detracted from its value as a sign of cancer, although cancer is rarely mistaken for tuberculosis. The only cancer cases that gave negative results were those in which the cancer had been excised. Murachi, also, found an increase in the neutral sulfur from cancer patients. The coefficient, according to him, may be 3.8 per cent of the total sulfur.

In contrast to the foregoing, Pribram found that only 60 per cent of cancer patients gave a positive Salomon-Saxl test, that the test is, therefore, far from specific. Alekseev came to a similar conclusion. Mazzitelli has studied this test in 50 cases of cancer, with and without cachexia. Of 18 cases of the latter variety, the test was positive in 14; but also in 8 of 10 cases of tuberculous cachexia, and 16 or 23 cases of cachexia of various origins, including 11 with cancer and 4 with tuberculosis; Greenwald concluded that this test has no value in the diagnosis of cancer.

The procedure for carrying out these tests is as follows: Of course, the specimen of urine examined is a twenty-four hour collection, preserved with about 5 grams thymol.

Salkowski-Kojo Test.—The urine was first tested for coagulable protein, which, if found, was removed by means of heat coagulation, with addition to the boiling liquid of a few drops of dilute acetic acid sol. To 100 c.c. of mixed, filtered, 24 hour specimen of urine, zinc sulfate was added in sufficient quantity to effect saturation. The saturated liquid was allowed to stand for 24 hours, then was filtered through ashless paper, and the precipitate washed several times on the paper with saturated zinc sulfate solution, to remove nitrogenous substances adherent to the precipitate. The paper and precipitate were then placed in a Kjeldahl flask and the nitrogen content determined by the Kjeldahl method. The total nitrogen in 5 c.c. of urine was also determined by the Kjeldahl method. The ratio of the nitrogen in the zinc sulfate precipitate to the total urinary nitrogen was computed.

Salomon-Saxl Test.—The technic of the Salomon and Saxl neutral-sulfur test is the following: 150 c.c. of urine, freed from coagulable protein by heat and acid, are diluted with 100 c.c. of water. A mixture of 100 c.c. of sat. aqueous sol. of barium hydroxid and 50 c.c. of sat. aqueous sol. of barium chlorid is added, the liquid filtered, and the filtrate tested with barium to see if precipitation is complete. In order to remove the ethereal sulfates, 300 c.c. of the filtrate are treated with 30 c.c. of conc. hydrochloric acid sol., and boiled for 15 min. in an Erlenmeyer flask, using a funnel condenser. The flask is then placed on a water-bath for 24 hours. Of the clear filtrate, 200 c.c. are mixed with 3 c.c. of hydrogen peroxide (perhydrol-Merck), and boiled for 15 min. with a funnel condenser. After boiling, the liquid is transferred to a conical graduate, where, at the end of 6 hr., the amount of precipitate is observed. Antipyrin and creosote medications interfere, according to certain authors, with this test.

Goodridge and Kahn concluded from their observations that positive results with either the colloidal-nitrogen test (Salkowski-Kojo) or the neutral-sulfur test (Salomon-Saxl), alone, are not indicative of carcinoma. When performed conjointly on urine of the same case, however, positive results with both methods are strongly indicative of malignancy. The appended table extracted from the work of Goodridge and Kahn, is representative of the figures they obtained.

No.	Name	Diagnosis	Total N in 100 c.c. urine gm.	Colloid-N in 100 c.c. urine gm.	Per cent Colloid-N of Total N	Total S in 100 c.c. urine gm.	Salomon-Saxl neutral S in 100 c.c. urine gm.	Per cent Neutral S in Total S
1	A. I.	Normal	0.7459	0.01006	1.35	0.112	0.0019	1.72
2	A. I.	"	0.7875	0.0098	1.25	0.109	0.0018	1.65
3	J. S.	"	0.8132	0.0109	1.84	0.097	not w'g'd	less than 1
4	M. K.	"	0.7986	0.0167	2.10	0.124	0 0027	2.07
5	D. F.	"	0.9178	0.0164	1.74	0.171	0.0033	1.94

No.	Name	Diagnosis	Total N in 100 c.c. urine gm.	Colloid-N in 100 c.c. urine gm.	Per cent Colloid-N of Total N	Total S in 100 c.c. urine gm.	Salomon-Saxl neutral S in 100 c.c. urine gm.	Per cent Neutral S in Total S
23	T. A.	Cancer of uterus	0.9756	0.0419	4.3	0.085	0.0031	3.7
24	M. W.	Gastric cancer	1.1071	0.0636	5.75	0.087	0.0035	4.1
25	F. C.	Gastric cancer	1.1950	0.0652	4.62	0.108	0.0041	3.8
26	A. R.	Cancer of breast	1.2104	0.0568	4.7	0.152	0.0045	2.9
27	S. G.	Gastric cancer	0.9260	0.0361	3.9	0.095	not w'g'd	less than 1
28	T. S.	Cancer of liver	0.5762	0.0247	4.3	0.104	0.0035	3.4
29	T. A.	Cancer of uterus	1.3550	0.0469	4.2	0.087	0.0032	3.7
30	M. W.	Gastric cancer	0.8722	0.0305	3.5	0.1055	0.0025	2.5
31	S. G.	Gastric cancer	0.9128	0.0447	4.9	0.1243	0.0045	4.4
32	A. R.	Cancer of breast	1.0424	0.0458	4.4	0.1175	0.0037	3.2
33	A. G.	Cancer of rectum	0.4728	0.0212	4.5	0.1480	0.0050	3.4
34	C. J.	Cancer of cervix	1.1307	0.0431	4.7	0.0875	0.0030	3.5
35	W. J.	Gastric cancer	0.9246	0.0388	4.2	0.0986	0.0036	3.7
36	B. M.	Cancer of liver	1.1108	0.0377	3.4	0.097	0.0031	3.2
37	K. B.	Cancer of liver	0.8229	0.0427	5.2	0.1452	0.0062	4.3
38	E. F.	Cancer of stomach	1.1055	0.0608	5.5	0.1445	0.0059	4.1
39	B. J.	Cancer of pancreas	1.1782	0.0494	4.2	0.1378	0.0060	4.4
40	E. M.	Cancer of stomach	1.1363	0.0441	3.8	0.1025	0.0043	4.2
41	B. M.	Cancer of liver	0.8912	0.0427	4.8	0.1644	0.0070	4.0
42	C. J.	Cancer of cervix	0.7755	0.0334	4.3	0.1552	0.0067	4.5
43	P. B.	Cancer of uterus	0.5737	0.0252	4.4	0.1275	0.0049	4.1
44	M. G.	Cancer of stomach	0.9345	0.0345	3.7	0.09865	0.0035	3.4
45	E. F.	Cancer of stomach	0.8548	0.0435	5.1	0.1143	0.0038	3.5
46	M. W.	Gastric cancer	0.9845	0.0502	5.1	0.0975	0.0026	2.7
47	F. O.	Cancer of pelvis	0.9642	0.0424	4.4	0.0956	0.0039	4.2
48	M. F.	Cancer of cervix	0.8750	0.0411	4.7	0.1140	0.0031	2.9
49	C. J.	Cancer of cervix	0.6437	0.0270	4.2	0.1231	0.0037	3.1
50	R. W.	Cancer of rectum	0.8821	0.0379	4.3	0.1242	0.0041	3.4
82	L. S.	Lung tbc.	0.872	0.0117	1.35	0.1409	0.0047	3.4
83	A. H.	Nephritis	0.695	0.0118	1.75	.....	not w'g'd	less than 1
86	C. H.	Myocarditis	1.078	0.0363	3.4	.....	" "	" " "
89	S. E.	Typhoid	0.9465	0.0118	1.25	.....	" "	" " "
92	B. R.	Empyema	0.8253	0.0139	1.75	.....	" "	" " "
95	H. W.	Endarteritis obliter.	0.7321	0.0102	1.4	.....	" "	" " "
97	M. R.	Endarteritis obliter.	1.0878	0.0097	0.9	.....	" "	" " "
98	J. R.	Sarcoma of leg	1.046	0.0468	4.5	.....	" "	" " "
99	C. S.	Leukemia	1.0975	0.0239	2.2	.....	" "	" " "
100	Child	Hemophilia	0.784	0.0109	1.4	0.1482	0.0033	2.4
101	C. F.	Pernicious anemia	1.095	0.0130	1.2	0.1843	0.0045	2.5
102	R. K.	Atrophic cirrhosis	0.5965	0.0106	1.8	0.1077	0.0029	2.8
104	A. E.	Pneumonia	0.6546	0.0111	1.7	.....	not w'g'd	less than 1
105	R. E.	Pneumonia	1.0725	0.0161	1.5	.....	" "	" " "
108	J. M.	Diabetes	1.0953	0.0466	4.25	.....	" "	" " "
109	J. A.	Diabetes	1.2075	0.0465	3.75	.....	" "	" " "
110	B. S.	Diabetes	0.9642	0.0501	5.2	.....	" "	" " "
113	A. K.	Syphilis	0.7114	0.0263	3.7	.....	" "	" " "
114	H. H.	Syphilis	0.7227	0.0296	4.1	.....	" "	" " "
115	S. H.	Syphilis	0.5835	0.0222	3.8	.....	" "	" " "
116	M. F.	Gastric ulcer	0.6444	0.0077	1.2	.....	" "	" " "
117	M. M.	Gastric ulcer	0.9007	0.0126	1.4	0.1586	0.0036	2.4
118	B. W.	Gastric ulcer	0.8767	0.0075	0.85	0.1755	0.0042	2.5
119	C. Z.	Gastric ulcer	0.6114	0.0109	1.7	.....	not w'g'd	less than 1
124	P. B.	Endocarditis	1.0755	0.0258	2.4	.....	" "	" " "
127	H. F.	Lung tbc.	1.2005	0.0281	1.4	0.1722	0.0063	3.7
128	N. K.	Lung tbc.	0.9234	0.0139	1.5	0.1645	0.0046	2.9

Since 1914 we have had occasion to perform these tests for malignancy in a large number of cases. From our statistics we conclude that a positive result with both the Salkowski-Kojo and Salomon-Saxl Test is very suspicious of carcinoma.

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THE UTILIZATION OF DEXTROSE IN THE ANIMAL BODY*

By J. J. R. Macleod, M.B., Cleveland, Ohio.

THE common practice for determining the assimilation limit of dextrose consists in giving a weighed quantity of this sugar by mouth on an empty stomach and thereafter testing the urine at intervals for its presence. When the urine is allowed to collect for twenty-four hours, the mixed specimen shows no trace of dextrose in the majority of healthy individuals after a dose of 200 gm.; after 300 gm. a somewhat higher percentage of cases develop a mild glycosuria, but frequently no trace becomes evident even after 500 grams. Beyond the last mentioned amounts, the limit of ingestion is reached, on account of nausea, etc., and it is improbable that, even if larger amounts could be tolerated, any more of the dextrose would be absorbed than with 300 or 400 grams.[1] It is likely that glycosuria would have been detected in the above tests had the urine been examined at frequent intervals after taking the dextrose instead of examining a mixed specimen, but even granting this to be the case, it is clear that the assimilation limit can signify practically nothing regarding the ability of the organism to metabolize carbohydrate.

These observations have an important bearing on the now well-established practice used for the detection of incipient (so-called "latent") cases of diabetes. Following the suggestions of the von Noorden school, it is usual to consider that a patient is to be thus classified if dextrose appears in the urine after alimentary ingestion of 100 gm. dextrose on an empty stomach. In the light of the above results and for many other reasons, there is considerable doubt as to the value of this test. Thus, when a solution of dextrose is given orally, its rate of absorption will depend very largely on the motility of the stomach. If this be normal, the solution will very quickly find its way past the pyloric sphincter into the intestine, where it will be rapidly absorbed. If on the other hand the pyloric sphincter should not open freely, the passage of the dextrose into the intestine may be so delayed that no more is present in this place at one time than would be the case after an ordinary diet of polysaccharide. And even after the sugar solution enters the small intestine differences in the amount of the intestinal contents with which it becomes mixed, in the extent of bacterial growth, and in the absorption process, may very materially affect the rate at which the dextrose gains entry to the blood.

Recognition of these facts has led those who have desired to determine the assimilation limit in laboratory animals to administer the dextrose intravenously. But even this refinement in technic has not, as a rule, had the effect of rendering the results of any very evident value as a criterion of the utilization of dextrose in the animal body. The reason for this unreliability of the method is mainly that the period of injection of the dextrose solution usually occupies only a few minutes, so that it causes a sudden, instead of a very gradual increase in the sugar concentration of the blood, the conditions being quite unlike those which exist during the normal absorption of dextrose from the intestine. The

*From the Laboratory of Physiology, Western Reserve Medical School.

mechanism by which the body ordinarily disposes of excessive amounts of dextrose, absorbed into the portal blood, is not adjusted to operate when the systemic blood is s ddenly overcharged with this substance. In the one case the dextrose is a foodstuff; in the other, because of its excessive concentration in the blood, it is more or less of a poison. Such results, in other words, merely show us how much dextrose can be added at one time to the organism without any overflow taking place into the urine, but they furnish us with no information regarding the power of the organism to utilize a constant though moderate excess of this substance. In the one case it is the "saturation limit," in the other the "utilization limit" of the organism for dextrose, that we are really considering (Blumenthal[2]).

Consideration of these principles has led Woodyatt, Sansum and Wilder[3] to undertake a thorough reinvestigation of the whole problem of the utilization or, as they prefer to call it, the tolerance of the body for dextrose. They emphasize the obvious fact that the ability of the organism to utilize dextrose "must depend on the rate at which the tissues are able to abstract it from the blood by their combined powers, to burn it, to reduce it into fat or to polymerize it into glycogen." To form any estimate of the combined effect of these processes, we must take into account not only the amount of dextrose per unit of body weight (grams per kilo.), but also the rate of injection, for "tolerance must be regarded as a velocity, not as a weight." Woodyatt and his co-workers have devised a very satisfactory apparatus by which such injections can be made at a regular, definite rate over prolonged periods of time. We have used this apparatus extensively in this laboratory, and can vouch for its perfect reliability. It is being used now in clinical practice wherever continuous injection, not only of dextrose but of any other fluid (e.g., of bicarbonate solutions in cases of acidosis) is desired.

Briefly summarized, the conclusions which Woodyatt, etc., have so far drawn from their investigations are as follows: In a normal rabbit, dog or man, 0.8-0.9 gram. of glucose per kilo. body weight and per hour can be utilized by the organism for an indefinite time without causing glycosuria. When between 0.8 and 2 grams are injected, a part of the excess appears in the urine, steadily increasing until a maximum is reached, after which the excreted fraction remains constant (at about one-tenth). If more than about 2 grams per kg. and hr. are injected, "a large percentage of all glucose in excess of the 2 grams per kg. and hr. appears in the urine, once constant conditions are established."

Being thus furnished with accurate information regarding the tolerance of the organism for dextrose, the question naturally presents itself: how is the dextrose disposed of? As indicated above, three means of disposal are available: conversion into glycogen, oxidation into carbon dioxide and water, and reduction into fat. It is commonly assumed that the operation of these processes is pretty much in the order named; that is, the excess of dextrose is first of all dealt with by conversion of the excess into glycogen, which process is soon followed, if not accompanied from the start, by an increased combustion of dextrose in the tissues and then, if there still be an excess to deal with, by the reduction of some of the carbohydrate to fat. Important investigations have

recently been published by several workers (Murlin and Kramer,[4] Verzár,[5] etc.), regarding the burning of the excess of dextrose, but as to the mechanism involved in its polymerization into glycogen or its reduction to fat, little is as yet known.

From the point of view of diabetes, it is obviously important that we should pay particular attention to the glycogenic mechanism, for it is the first line of defense against the invasion of the tissues by an excess of dextrose, and it is presumably only after this barrier is broken through that there is danger of the whole defensive system of the body against such an excess becoming disorganized. A fundamental question regarding this first line of defense is of course concerned with its disposition; in what parts of the body is it best developed? There are theoretically two methods by which such a problem could be investigated: first, by comparing the amount of glycogen in the various parts of the body before and after the continuous administration of dextrose, and secondly, by comparing the percentage of dextrose in the blood flowing into and out of the organs. Of these, the first mentioned method has been extensively used and the results have shown that by far the largest storage of glycogen, per unit of tissue, occurs in the liver, and the next largest in the muscles, in which however the distribution is by no means uniform, constantly active muscles such as the heart and diaphragm, being especially rich in glycogen. As judged then from the concentration of glycogen which may be present, the liver would appear to be the strongest line of defense, and the position of this organ in relationship to the usual path of invasion of dextrose would indicate that it is also the most advanced.

If instead of the concentration, we consider the total storage capacity (for glycogen) of the liver as compared with that of all the muscles, we find it to be at least potentially greater in the muscles than in the liver, so that the question naturally presents itself as to the circumstances under which this muscle storage will be called into operation. It can only be the dextrose which escapes the liver and gains entry to the systemic blood that the muscles deal with; but, to what extent does this occur? Is the glycogenic mechanism of the liver so efficient that it holds back most of the absorbed dextrose until the hepatic cells become overloaded with glycogen, or does the liver only hold back some of the excess of dextrose in the portal blood and allow the rest to pass on? As judged from the results which have been published in recent years bearing on the behavior of the percentage of dextrose in the blood of the systemic circulation during the alimentary ingestion of dextrose (Böe,[6] etc.), it would appear that much of the dextrose passes through the liver, thus causing a post-prandial hyperglycemia and indicating that the liver barrier is much less efficient than had previously been thought to be the case.

* * * * * *

To throw further light on the problem, we have been engaged for some time in an investigation of the comparative dextrose-storing abilities of the liver and muscles as judged by the second of the possible methods indicated above, namely, by a comparison of the dextrose concentration in the inflowing and outflowing blood of the liver and muscles. Theoretically, such a plan of investigation would seem to be ideal in furnishing us with the information we desire,

but in practice the interpretation of the results is considerably obscured on account of the enormous volumes of blood which pass through these organs, thus making it possible that considerable quantities of dextrose might be removed from or added to them without any very definite change occurring in the percentage of dextrose in the blood. Thus, in a dog of average size, 6 kg., about 400 c.c. of blood traverses the liver in one minute. If the percentage of dextrose should fall from 0.200 in the blood of the portal vein to 0.175 in that of the hepatic veins, it would mean that in one minute 0.1 gm. of dextrose had been retained, or 6 gm. an hour, or 1 gm. per kg. per hr. That is to say, with the amount of dextrose just over the tolerance limit of 0.8 gm. per kg. and hr., and assuming that all of the absorbed dextrose was retained by the liver, there would be a difference between the percentages of dextrose in the portal and hepatic vein bloods of only 0.025, which, although well beyond the experimental error of the methods now commonly used for sugar estimation in blood, is nevertheless far from striking.

The experiments were performed on etherized dogs that had been fed for some days previously on a mixed diet of bread and flesh. The liver and muscles would therefore contain a moderate amount of gycogen. By a technic, the details of which are described elsewhere, cannulæ were then placed in the branches of the portal vein, inferior vena cava, and iliac vein; and through these cannulæ small measured quantities of blood (2 c.c.) were removed at frequent intervals, and the sugar percentage determined by Pearce's modification of the Lewis-Benedict method[7]. Another cannula was inserted into one of the smaller branches of the mesenteric vein, through which the solution of dextrose was injected. To maintain the injection at a constant rate, the cannula was connected either with a constant pressure perfusion flask or with the Woodyatt apparatus.

In order to allow as much as possible for the slight differences in blood-sugar concentration which are to be expected, even with active glycogen formation, the blood samples were removed at frequent intervals (3-10 minutes), and curves plotted with the percentage of dextrose along the ordinates and time intervals along the abscissa. If the curves thus drawn for the portal vein and vena cava should coincide, it would indicate that no detectable glycogen formation had occurred in the liver; and similarly, if the same were the case for those of cava and iliac vein, that the muscles of the corresponding hind limb had retained none of the dextrose. Want of correspondence of the curves need not however mean that sugar retention or liberation has occurred, for the same results would be obtained when water was added to or removed from the blood. To control this source of confusion in the interpretation of the results, enumeration of the corpuscles or estimation of the hemoglobin should have been made. The already existing complexity of the experiments, however, made it impossible for us to do this.

The results will be published in detail elsewhere, but a few of the most important conclusions which may be drawn from them will be indicated in a preliminary way here. When the dextrose is injected in amounts that are below the tolerance limit of the animals (0.8 gm. per kg. and hr.), a more or less rapid increase occurs in the blood-sugar until a certain level is attained, after which the percentage remains constant. The time required for this level, or plateau,

to be reached is definitely shorter with small than with large injections; thus, the level was reached in about 25 minutes with 0.4 gm. dextrose per kg. and hr., and 35 minutes with 0.7 gm. These facts indicate that the sugar-retaining powers of the organism very quickly adjust themselves to the increased work thrown on them by the addition of dextrose to the blood. Utilization soon becomes so adapted that the dextrose is removed from the blood as quickly as it is being added, so that after the plateau has been gained, the rate of disposal of dextrose by the organism may be determined by the rate at which the injection is being made. This gives us a valuable test object on which to investigate the influence of various conditions (such as injections of acids and alkalies, drugs, stimulation of nerves, etc.) on sugar utilization. We shall return immediately to some results of this nature, but meanwhile it is important to note that comparisons of the sugar percentage in the blood of the above mentioned veins did not show any constant differences to exist, so that we cannot conclude from them whether the liver or the muscles contributes the more to the removal of the excess of dextrose, when this excess is of small magnitude.

These results prompted us to repeat the investigations using injections of dextrose that were decidedly above the utilization limit. Under such conditions it was thought that the ability of the liver and muscles to form glycogen would become stimulated to such a degree that differences in the concentration of sugar in the inflowing and outflowing blood would become apparent. Three such experiments, each occupying several hours, were performed, the rate of injection being respectively 2.4 gm., 3.0 gm. and 3.5 gm. dextrose per kg. and hour. In all three cases the blood-sugar curves rose steadily throughout the injection periods, there being no tendency, as in the previous experiments to the establishment of a plateau. In the experiments in which 2.4 and 3.5 gm. dextrose were injected, the blood-sugar curve was decidedly highest in the portal vein, next in the vena cava, and lowest in the iliac vein, the differences between them being about equal, thus indicating, as far as can be judged from such results, that the sugar-retaining powers of the liver and combined muscles of the hind limb are equal. In the experiment in which 3.0 gm. dextrose was injected, however, the curve for the blood of the iliac vein stood constantly at a higher level than that of the vena cava, which on the other hand was distinctly lower than that of the portal vein. On superficial examination such a result would seem to indicate that, instead of retaining some of the dextrose, the muscles had actually been contributing dextrose to the blood, a conclusion which it is unlikely can be correct. A much more probable cause for the anomalous result is that the blood had lost water during its passage through the muscles, and this loss of water was probably to be accounted for by the fact that considerable quantities of hydrochloric acid were injected at frequent periods along with the dextrose. Unfortunately in this experiment neither the H-ion concentration of the blood nor the number of corpuscles in it was determined, so that we cannot be certain that our explanation of the results is correct.

These observations on the effect of large injections of dextrose are of especial interest, because it has been by such experiments that several investigators have sought to throw light on the problem of the disposal of dextrose in the animal body. The most recent work of this nature is that of Kleiner,[8] who

found that after the injection of large amounts of dextrose (4 gm. per kg. body weight) intravenously, the blood-sugar suddenly became enormously increased and then immediately began to fall, reaching the normal again in about 90 minutes. It was found that although some of this excess is drained away in the urine, the amount must be small, since as rapid a disappearance of the excess of sugar occurred in dogs from which the kidneys had been removed as in normal animals. In another series of animals, the aorta and vena cava were tied just above the diaphragm, so as to exclude the liver from the circulation, and it was found that the injected sugar disappeared just as quickly as in intact animals. From this result Kleiner concludes that conversion by the liver of some of the excess of sugar into glycogen cannot be an important factor in the disappearance, but that absorption by the muscles must be mainly responsible. By examination of the amount of sugar in the muscles, a great increase was found, some of the absorbed dextrose being present in a polymerized state. There is, however, one serious objection to all experiments of this nature, namely, that by the sudden addition of excessively large amounts of dextrose to the blood at one time, conditions become established which can never obtain in even the severest types of experimental diabetes. Not only will the osmotic relationships between the blood and tissue fluids be entirely upset, but toxic influences dependent either directly on the excess of dextrose itself, or indirectly on the appearance of decomposition products (e.g., lactic acid) produced from it, will come into play, and almost certainly throw out of working order those mechanisms which under ordinary circumstances would take care of any physiological excess of dextrose in the blood.

The main difference between our own and Kleiner's results is that we have found the liver to be capable of removing some of the excess of dextrose, whereas Kleiner did not. It might be raised as an objection to our results that the lower percentage of dextrose in the blood of the vena cava over that in the portal vein was merely due to the dilution which the blood, flowing into the cava from the liver, suffered on mixing with the blood meanwhile ascending from the leg veins and to its dilution by the blood of the hepatic artery. Although we are prepared to admit that such dilution may account for part of the decrease, we feel confident that it is an unimportant factor because: (1) the veins of one kidney and one hind leg were ligated, and (2) in one of the three experiments of this group there was a higher percentage of sugar in the muscle-blood than in that of the vena cava opposite the liver.

* * * * * *

As has been indicated above, one object of the above observations was to secure suitable experimental conditions upon which to investigate the influence of various factors on the assimilation of dextrose. Of these we have so far investigated the influence of alterations in the reaction of the blood.

Before proceeding to indicate the general nature of the results, it may be well to point out that there has been an accumulating mass of evidence during recent years tending to show that the behaviour of carbohydrate in the animal body is decidedly influenced by the reaction of the tissue fluids. Thus, in dyspnea, where the H-ion concentration of the blood becomes increased, hyper-

glycemia and glycosuria are commonly observed, and after the administration of acids by stomach the assimilation limit of dextrose becomes much depressed. Hyperglycemia is also more readily established when acid and dextrose are ingested than when a corresponding quantity of dextrose is given alone, and glycogen accumulates in the liver to a much less degree (Elias[9]). On the other hand, when alkalies are administered intravenously to normal rabbits, a decrease frequently occurs in the percentage of blood-sugar (Underhill[10]), and when given to dogs rendered diabetic by extirpation of the pancreas, the glycosuria almost, if not entirely, disappears (Murlin and Kramer[1]). Furthermore, adrenin does not cause so marked a degree of hyperglycemia when it is administered to rabbits following the injection of sodium carbonate as it does in normal animals (Underhill).

In view of these results the question arises: upon what sugar-disposing function of the body does the acid or alkali exert an influence? Two possibilities are to be thought of: it acts either (1) on the storage (polymerization) of sugar as glycogen in the liver and muscles, or (2) on the oxidation of sugar in the tissues. To throw light on this problem Murlin and Kramer examined the respiratory quotient, $\frac{CO^2}{O^2}$, of normal and diabetic dogs under the administration of sodium carbonate and dextrose. An increase in the quotient (towards 1.0) indicates increased combustion of dextrose, and although such was not found to occur when alkali was given to a normal animal, it did occur when alkali was given intravenously along with dextrose to one that was diabetic. The conclusion drawn from these results is that the alkali stimulates the oxidation of dextrose in the tissues, a process which, however, might be taking place at the same time as one of a greater storage of other molecules of dextrose as glycogen. Kramer and Marker[11] have however recently contributed evidence which would seem to indicate that such increased glycogen formation does not occur under the above conditions. These authors found that glycogen did not appear in the liver following the administration of the alkali, nor by giving adrenin to a depancreated dog that had been retaining dextrose while on a meat diet, were they able to cause an increased excretion of dextrose in the urine.

These very significant observations concerning the beneficial influence of alkali administration on that form of experimental diabetes which most closely simulates the more acute varieties of the disease in man prompted us to investigate, by the methods above described, whether or not sugar-storage by liver and muscles could be stimulated by injections of alkali (sufficient to perceptibly lower the H-ion concentration of the blood), and whether acids would have the opposite influence.

Details of our results will be published elsewhere, and we will accordingly only briefly offer a preliminary notice of some of them here. In the first place, it was noted that when neutral dextrose solutions are injected intravenously, there is a distinct tendency for the H-ion concentration of the blood—as measured by the colorimetric method—to become increased. This is possibly due to the production of decomposition products of the dextrose (lactic acid); at least we have been able to show that the blood of such injected animals contains about twice as high a percentage of lactic acid as that of normal animals. Even

when, along with the dextrose, moderate quantities of sodium carbonate are also injected, there may be distinct (colorimetric) evidence of increasing blood acidity, so that to really produce an alkalosis, when dextrose is being injected, very large injections of alkali are necessary. When enough alkali was injected to distinctly depress the H-ion concentration of the blood, it was usually found that the liver more actively removed sugar from the blood flowing through it than ordinarily. By injecting a sufficient amount of alkali into dogs whose blood already contained an excessive concentration of dextrose, as a result of previous feeding with excess of carbohydrate and operative manipulation, a distinct lowering of the blood-sugar concentration became evident, but it could not be shown, by comparison of the curves representing this in the portal vein, vena cava and iliac veins, whether the liver or muscles were mainly responsible for the disappearance.

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PITUITARY STANDARDIZATION*

By H. C. Hamilton and L. W. Rowe, Detroit, Mich.

THE value of pituitary extract (posterior lobe) as an effective therapeutic agent for controlling blood pressure and for stimulating uterine contractions in the second stage of labor is universally recognized. Because of these important uses it is necessary that the extract be standardized as accurately as possible.

The crude materials from which valuable substances are obtained are unfortunately very variable. This, however, is of slight importance compared to the effect that the various chemical and pharmaceutical manipulations, incident to the preparation of these substances, have on their activity. The adoption of a high standard of activity and rigid adherence to the same is imperative for a product of such great physiologic importance as that of pituitary extract. In the case of this extract, as is also true of extracts of some other glands and crude drugs, the chemical constituent to which it owes its therapeutic value has not been isolated.

In such cases advantage is taken of the fact that active medicinal substances when administered to animals have characteristic physiologic effects which are more or less typical and which can be used as assay reactions. This procedure is similar to the use of chemical reactions on which to base chemical assay processes. Biologic standardization is now generally recognized as an indispensable adjunct to the commercial valuation and to the scientific investigation of certain substances of great importance in medicine.

When several physiologic effects are more or less characteristic of the substance in question, differences of opinion naturally prevail as to which is best adapted to an accurate quantitative assay process.

It is the intention of the authors to describe the two methods that have been proposed and used for the assay of the various substances and extracts derived from the pituitary gland, and to point out the advantages and disadvantages of each as these have developed under practical working conditions.

The two methods referred to above are those known as the blood pressure and the oxytocic tests.

In a former communication[1] some of the physiologic effects following the administration of extracts of the pituitary body were reviewed and discussed as to their adaptibility to the purpose of standardization.

Of the various characteristic effects, that on the circulatory system was considered most constant and subject to the least variation due to causes other than differences in the amount administered. This effect was, therefore, adopted as an assay reaction to determine the activity of solutions prepared directly or indirectly from the infundibulum of the pituitary gland.

THE BLOOD PRESSURE METHOD.

The method of assay in brief is as follows: A dog weighing approximately 10 kilos is anesthetized, preferably with chloretone, and is prepared for test pur-

*From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.

poses by inserting a cannula into a femoral or other convenient vein for injecting the solution, and another cannula into one of the carotid arteries. By means of a flexible connection the artery is joined to a manometer attached to a kymograph, to obtain a record of the normal heart action and blood pressure for comparison with that following an injection of the active extract. The standard originally[1] used for comparison in making such assays was the activity obtainable from the dried, defatted, powdered, infundibular portion of the bovine glands. Gland material so prepared seems to retain its activity indefinitely and to permit of quick and complete extraction by simple infusion. Later, however, the possible objections to this have been obviated by the use of a stable and highly active water-soluble powder prepared by Aldrich.[2] This was carefully assayed and its activity compared with that of the former standard. The Standard Test Solution is prepared from this, using a sufficient quantity of the powder to make a solution containing in 1 c.c. the highest activity found in 1 mg. of the dried gland material referred to above.

The technic of the test is to inject the standard test dose—1 c.c. of the Standard Test Solution—into the vein, note the rise in blood pressure, and after 15 minutes repeat the injection. It is some times necessary to repeat this injection two or three times before one obtains an equal change in blood pressure from two consecutive injections of the test dose.

The standard and sample are then injected alternately with intervals not less than 15 minutes, varying the amount of the sample, if necessary, until the rise in blood pressure is the same from definite quantities of each. The activity of the sample can be deduced from the amounts used and is stated in terms of the Standard.

The following points must be carefully observed as a means of making this an accurate quantitative method for the valuation of pituitary extracts:

1st. The dog must be healthy and should maintain a good normal blood pressure.

2nd. It must be completely anesthetized until all reflexes are destroyed.

3rd. It must be sensitive to changes of 10 per cent in the amount of pituitary extract injected. For example, if doses of 0.9, 1 and 1.1 c.c. of the test solution induce the same amount of change in blood pressure with no measurable differences between them, that dog is not in a condition satisfactory for standardization work.

4th. It must be sufficiently sensitive to pituitary extract so that 1 or at most 2 c.c. of the Test Solution (q.v.) raises the pressure at least 1 cm.

5th. Two consecutive injections should have at least 15 minutes interval between them.

The method just described was adopted tentatively until a more logical or accurate one could be developed.

THE ISOLATED UTERUS TEST.

The increasing use of pituitary extract in obstetric work, suggested a much more logical method of assay than the one described, namely, a test based on the action of pituitary extracts on the uterus muscle. Such a method was used and first described by Dale and Laidlaw[3] and afterwards by various investigators,

notably Führer[4] and Guggenheim.[5] The experimental work has been exhaustively summarized by Roth[6] who assayed eleven commercial samples and reported his results.

The great differences in activities of the samples examined led him to the conclusion that "the statement 'physiologically standardized' means practically nothing." Roth, however, apparently overlooked the fact brought out by his own tests that in every case the two samples from the same manufacturer had the same activity, indicating that each laboratory although having a different standard had adhered to it.

The fault, if any existed, was not in the lack of a standard nor in a failure to apply standardization tests, but in the failure of the manufacturers to cooperate in choosing a single standard.

Roth, in a previous article[7] proposed the use of β-iminazolylethylamine, commonly called histamine, as a standard for measuring the activity of pituitary extracts. His proposal now reappears as the official standard for a U. S. P. solution of hypophysis extract and is compulsory to the extent that extracts standardized in any other way do not meet official requirements. While a very dilute solution is required for standardization purposes, the present almost prohibitive price of histamine, due to the fact that the supply in this country is very limited, tends to preclude its use as a standard.

Such a standard is also open to question from two viewpoints. Are different lots of histamine equal in their action on the uterus? Is a substance which has no practicable value in obstetrics,[8] when used alone, a true standard for determining the activity of a powerful oxytocic agent such as pituitary extract, and is it a proper standard when the action in question is as a pressor agent? If the value of pituitary extract were only as an oxytocic agent or if only that portion applied to obstetrical purposes were to come under the U. S. P. ruling then some of the objections to the method and the standard would be removed. Under present conditions, however, a more commendable standard would be a substance prepared from the gland and having the same qualitative action as fresh glandular extracts.

While the details of the technic and equipment for this test vary in different laboratories, the essentials are briefly as follows: One horn of the excised uterus of a young virgin guinea pig is used, it being suspended between a fixed point, and the end of a movable lever. It is bathed in Locke's solution which is kept at a uniform temperature of 38° C and constantly aerated with air or oxygen.

The solutions to be tested are mixed with the Locke's solution in which the muscle is suspended and only the small part in contact with the strip of uterus is responsible for the effect produced. The exquisite sensitiveness of this muscle to the action of stimuli such as an active pituitary extract, is shown by the fact that some specimens are sensitive and respond to the stimulus of the infinitesimal amount which comes in contact with them from a solution containing only 1 part in 100,000,000. This shows too the exceedingly powerful character of the active principle of the gland, for although the substance referred to above was highly purified, it did not possess the physical characteristics of, and probably was not a pure principle.

Without entering into a detailed description of the method by the authors,

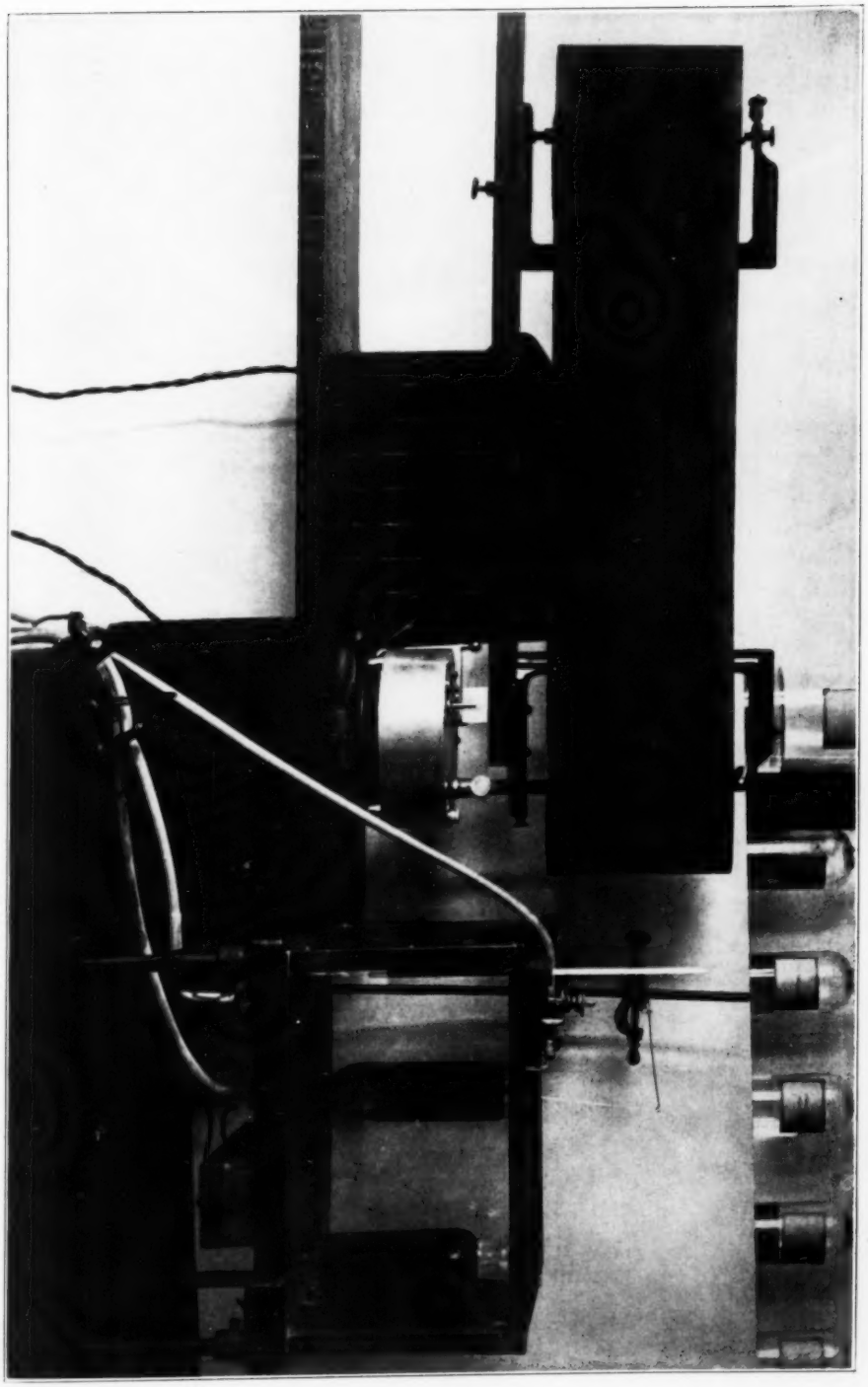

Fig. 1.—Complete equipment for oxytocic test.

which is outlined above, some points will be especially considered because so much depends on the carefulness with which they are followed.

The Test Animals.—While differences of opinion exist as to the one best adapted, most investigators have agreed on the young virgin guinea pig weighing 250 to 350 gms. and rejecting those in the œstral stage.

The Organ.—One horn of the uterus using a strip 1 to 2 cm. long. It should be normally slender and not congested. Congested, thickened uteri, while often very sensitive, are too irritable for standardization tests.

The Apparatus.—This includes the kymograph, a writing lever, a constant temperature bath accurately adjusted to hold a temperature of 38° C, an inner container for Locke's solution in which the uterus muscle is suspended, and facilities for preheating the Locke's solution to the same temperature as that of the bath, and for its aeration (see Fig. 1).

The heating of the constant temperature bath can be done accurately and conveniently by the method described by Dale and Laidlaw, a gas flame striking a copper rod which passes into and around the outer vessel. The apparatus in use at present and which has proved very satisfactory for maintaining a uniform temperature consists of a rectangular vessel of 8 liters capacity which is heated from below by an electric heating coil enclosed in a soapstone box. The amount of current is regulated by a rheostat and when once adjusted will maintain the temperature of the water bath at 38° C provided the room temperature remains fairly constant. The large outer vessel has a soapstone bottom covered inside with copper while two sides are made of double walled glass with an air space between. The apparatus is well adapted to the purpose intended and is very simple in construction and operation.

The preheating of Locke's solution is most conveniently done by keeping a supply in another container in the outer bath in which it attains and holds the same temperature as that in contact with the muscle. (Note bottle to right in large vessel.)

The aeration of the muscle is best secured by the method suggested by Dale and Laidlaw, conveying a current of air or oxygen through the tube (leading to top of inner container) which forms the lower support of the muscle, the upper end of the muscle being attached by thread or otherwise to the writing lever.

The writing lever should be light, such as a straw or an aluminum wire, should move almost without friction, and magnify the contractions 3 to 5 times.

Not of least importance is the Locke's solution which should be made of the best quality "reagent" chemicals.

The Technic.—This includes weighting the muscle to counteract the excessive contractions of an irritable uterus; injecting into the Locke's solution quantities of sample and standard which will induce equal contraction of the uterus less than the maximum for the specimen; maintaining in the meantime a constant temperature, removing spent pituitary solution; washing the specimen, and repeating injections not too often to destroy its tonicity. Injections can best be made by means of a hypodermic syringe which forces the dose into the solution and tends to mix and make the solution homogeneous almost immediately. This is aided by the continuous bubbling of air through the solution. When the

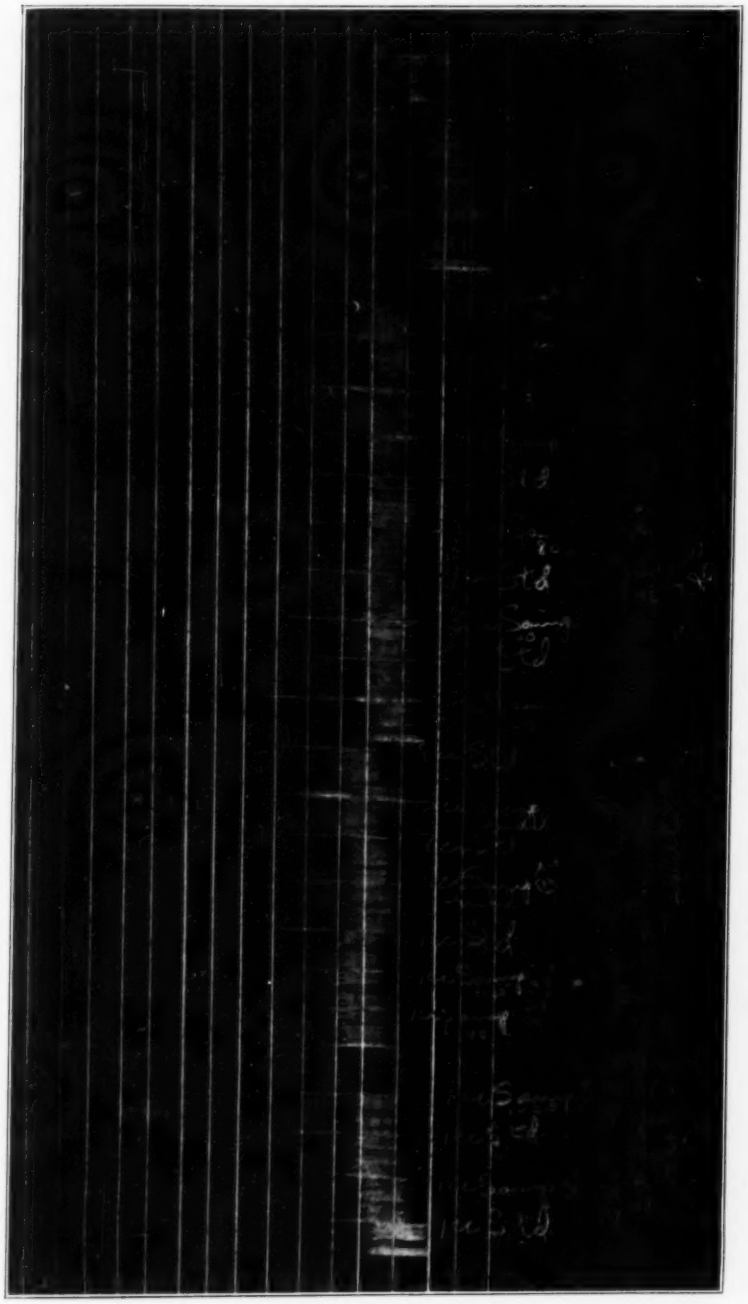

Fig. 2.—Twenty-four consecutive injections of pituitary extract into the same dog.

maximum contraction for any dose has been reached and no further rise of the writing lever takes place, the spent solution is withdrawn through a valve at the bottom of the inner container passing through the bottom of the outer bath. The specimen is washed with fresh Locke's and an additional quantity is left in contact filling the container to some determined volume. Another injection can be made as soon as the muscle has relaxed to normal length.

DISCUSSION OF METHODS.

The oxytocic test on the uterus muscle is without question more logical than the blood pressure effect for standardizing substances used almost exclusively as oxytocic agents, unless there is evidence that the same active constituent is responsible for both effects. If this is true in any particular instance, then accuracy and convenience only need be consulted. Opinions differ regarding this point as applied to the pituitary gland but the most generally accepted one is that there are several active bodies with different physiologic actions. In other words an extract of the gland might raise the blood pressure without having any oxytocic activity. This is a point which requires more than passing consideration. Because of the fact that a good specimen of the isolated uterus of a guinea pig is sensitive to a much smaller quantity of pituitary extract than can be detected by the blood pressure test and that such a test organism is also very sensitive to other substances and to variations in the test conditions, it is very difficult to prove that there is both a pressor principle and an oxytocic principle in the glands. The authors have not yet found any data to support the contention that the two actions are not due to an identical substance. Any chemical treatment to isolate one from another either destroys both actions or affects them equally.

Another objection urged against the blood pressure method as of noteworthy importance,[6] is the statement that repeated injections of pituitary extracts, even when prepared from the separated infundibulum, induce progressively decreasing pressor effects until the depressor predominates and no rise in blood pressure follows the injection of the active extract. This, however, is not a valid objection when the test conditions outlined above are followed carefully.

In many cases between 20 and 30 doses of 0.05 c.c. Pituitrin or an equivalent quantity of similar preparations have been injected intravenously into dogs without the depressant effect becoming noticeably increased. (See tracing, Fig. 2.) If large doses were used and the injections followed each other closely the objection noted would be a valid one. All physiological testing, however, must be done under rigidly prescribed conditions, otherwise there is little accuracy.

In this method of testing, the test animal is the dog admitted by all to be the least subject to the so-called tolerance; the doses are small; they follow each other at intervals not less than 15 minutes; sample and standard are injected alternately and must give an equal rise in pressure, the rise in pressure from the standard test dose must be 10 mm. or more. Under such conditions the method is satisfactory.

McCord[9] demonstrated that the isolated organs except the heart respond almost without fatigue to repeated injections of pituitary extracts, the effect in nearly every case being markedly constricting on the muscle walls. The heart was depressed and in a very large majority of the experiments there resulted a

decreased rate and amplitude. These results seem to indicate that the pressor effect on the vessels is fairly constant, and discredit any theory of a pressor principle distinct from a depressor principle.

This is corroborated by Roth[6] in the statement: "By the blood-pressure method, a comparison could easily be made between Sample 1 (Pituitrin, P.D. & Co.,) and Sample 3 (Infundin) since the pressor response was little diminished even after hours when weak dilutions were used."

Roth further states, however, "On the other hand, it was very difficult to compare Samples 6 (Hypophysin) with Sample 1 (Pituitrin, P.D. & Co.), and impossible to compare Sample 8 (Pituitary Ext. Schering) with Sample 1." This statement has not been verified because if each is injected in sufficient quantity to give equal heights of blood pressure, the activities are certainly in inverse ratio to the dilutions used. If a preparation fails to raise the blood pressure, but with a dose 7½ times as large as another sample, has an equal effect on the isolated uterus, who can say that the latter and not the former is the true index to its therapeutic value? If an extract is so poorly prepared that a large quantity is needed to raise the blood pressure measurably, deleterious substances may be present in such a proportion as to obscure the pressor effect. However, would not these other substances have an equally obscuring effect on the oxytocic test?

The foregoing statements regarding the objections raised against the blood-pressure method are not intended to be taken as refuting them but to explain why the authors do not consider them so vitally important and to lay the facts open for general consideration. The fact that the oxytocic test and the comparison with histamine as a standard are now incorporated in the 9th Revision of the United States Pharmacopeia would indicate that these objections have been either answered or overlooked, possibly the latter since those most interested in the subject were ignorant of this contemplated step until it was accomplished.

The isolated uterus test, while in some respects logical, is not in its present form practicable to be used as a commercial method for the final standardizing of pituitary extracts. It is a good qualitative test but lacks the accuracy and dependability necessary for commercial quantitative standardization.

While apparently simple both in technic and in the apparatus needed, experience has proved that in the former, at least, it is quite the contrary; the large number of pigs that are not adapted to the test, the slow and unsatisfactory preliminary standardization of the uterus, and the inaccuracy of the test when applied to weak extracts are objectionable features as great as, if not greater than, the objections advanced against the blood-pressure method.

In attempting to apply this method there were weeks at a time when some unfavorable conditions prevented our obtaining results allowing any degree of comparison. Compare also Dale and Laidlaw (loc. cit.).

In some cases the uterus is so sensitive as to respond beyond the limits of measurement to minute quantities of an extract. Weighting the lever to overcome part of this sensitiveness often results in killing the sensitiveness even when this is done with the greatest care.

On some occasions (and this is the most frequent cause for rejecting a

specimen) it is impossible to obtain two equal consecutive contractions of the uterus from equal amounts of the same extract.

At times the return to normal after a contraction is so slow that whether one waits for spontaneous relaxation or attempts to counterbalance the contraction with weights or bring about relaxation by means of an adrenalin solution, the sensitiveness of the specimen is lost.

That the difficulties of the test have been discovered by every investigator is evidenced by the fact that there is little agreement among them in the description of the technic employed. Guggenheim claims that the rat is free from some of the objections charged to the guinea pig. Roth claims that the uterus of a virgin guinea pig is better than that from dog, cat or mouse. He also claims that for only about two hours can a uterus specimen be used for this work while Führer could use the same strip for a week.

The latest report on this point is that of Fenger, which appeared since writing this article,[10] who states that "The selection of a suitable uterus is not always an easy matter. Often three or four pigs have to be killed before a satisfactory strip is obtained. The individual sensitiveness towards pituitary and histamine varies considerably. It is, consequently, by comparing several tracings on different days that a fairly correct conclusion in regard to actual strength of a certain solution can be drawn. It would be advisable to obtain a standard which resembles more closely the active principle of the posterior lobe than does histamine."

With the strictest attention to details some of the objections have been minimized but when the test can not be made more accurate than merely to distinguish between dilutions, 1-1000, 1-2000 and 1-3000 [the middle one being considered equal to standard and the others credited with respectively greater and less contractions than standard (see reference 6)], its accuracy leaves much to be desired.

While apparently the oxytocic test on the uterus muscle is the more logical one for standardizing pituitary extracts intended for obstetrical use, one cannot but question whether it more certainly indicates oxytocic activity than does the blood pressure test. Several substances such as blood serum, beef bouillon, peptone, egg white, putrid meat extracts, act on the uterus muscle without having any recognized oxytocic value. [11, 12] In the case of pituitary extract, is it more than merely a coincidence that it acts on the excised uterus muscle and also on the gravid uterus? Agents with oxytocic value are, without exception, those which react on smooth muscular tissue; the arterioles and small veins, as well as the uterus, belong to this class of muscle. It is the action of pituitary extracts on these unstriated muscles which brings about increased blood pressure. This seems, therefore, to be a logical test reaction. In this connection another point should be given due consideration—the increasing use of pituitary extract for its blood pressure effect particularly during or after surgical operations. By its use the systemic pressure is increased while the pressure in the pulmonary circuit is lowered—a condition described by Wiggers[13] as "a fortunate combination of actions." Shall we apply the oxytocic test exclusively to a substance so valuable for its pressor effects?

From Roth's results[6] we tabulate the following comparisons of pressor and uterine values:

	Values.	
SAMPLE.	PRESSOR.	UTERINE.
1	15	7.5
6	1	1
8	?	1

On the face of this report it would seem possible for the obstetrician to determine whether Sample 1 is 15 times or only 7½ times as active as Sample 6. More striking still is the discrepancy in the case of Sample 8 of which Roth injected 250 times as much as of Sample 1 without an equal rise in blood pressure while the uterus test would indicate that it was not devoid of activity. This again is contrary to the statements of Dale and Laidlaw[3] that the pressor and oxytocic effects seem to go hand in hand, an observation made also by Frankl-Hochwart and Fröhlich[14] and repeatedly confirmed in our own work.

The problem seems to be one not for the pharmacologist alone, but for the clinician as well.

While we are not condemning as useless the uterus test for standardizing pituitary extracts, we are not willing to subscribe to the popular opinion that this test alone is deserving of recognition, that it has greater accuracy as a standard method of assay than the pressor test, or that the latter is not a measure of oxytocic activity.

We conclude, therefore:

First, that neither method in its present form is ideal as a means of standardizing pituitary extracts.

Second, that most specimens of the isolated guinea pig uterus are sensitive to the action of these extracts, some are exquisitely sensitive but few are sufficiently uniform in the reaction to be used for accurate standardization purposes.

Third, that the pressor test is a fairly accurate measure of pituitary activity, is not an illogical indicator of oxytocic value, and is free from some of the most objectionable features of the uterine method.

We regret our inability at the present time to add anything to the refinement of either method but suggest first, that the possibilities of the pressor test be further developed since it has been found capable in our hands of recognizing differences in pressor activity of less than 10 per cent; second, that chemists, pharmacologists, and clinicians work together in an effort to determine whether pressor and oxytocic values depend on the same active principle.

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LABORATORY METHODS

REVIEW OF THE FOLIN AND DENIS METHODS FOR THE DETERMINATION OF NITROGEN BY DIRECT NESSLERIZATION

By Roy G. Pearce, M.D., Cleveland, Ohio.

THE colorimetric methods for the determination of nitrogen in the blood and the urine have required that the ammonia produced by the destructive digestion with sulphuric acid or by catalyzers, such as urease, be removed from the digestive mixtures by aereation. This procedure besides requiring some time is difficult to carry through with certainty that all the ammonia is recovered, so that the publication of a method by Folin and Denis* for the direct Nesslerization of the ammonia in such mixtures is very welcome and of great value. Folin and Denis have described methods for the estimation of—(1) the total nitrogen in the urine, (2) urea nitrogen in the blood and urine, (3) urinary ammonia, and (4) the nonprotein nitrogen in the blood. The methods are briefly given here. The directions follow closely the concise descriptions given by Folin and Denis, and it is urged that they be followed accurately, since any alteration may lead to error, as the authors point out in the original paper.

TOTAL NITROGEN IN THE URINE.

The presence of large amounts of sulphates in the digestion mixture, following the destructive digestion of the urine with sulphuric acid was the chief obstacle in the direct Nesslerization of the ammonia contained therein. Folin and Denis have discovered that the presence of phosphoric acid in the digestion mixture not only hastens the oxidation but also allows direct Nesslerization of the ammonia.

SOLUTIONS AND APPARATUS REQUIRED.

Nessler Reagent.—This is prepared as follows: Seventy-five grams of potassium iodide are dissolved in 50 c.c. of warm distilled water and 100 grams of mercuric iodide added. The mixture is stirred until the solution is complete. The solution may be a little turbid. It is then diluted with four or five hundred cubic centimeters of water, filtered, and the filtrate made up to one liter. This is the stock solution from which the dilute Nessler reagent used in direct Nesslerization is made by adding to 300 c.c. of the above solution 200 c.c. of a 10 per cent sodium hydrate solution and 500 cc. of water. Fifteen cc. of this dilute Nessler's solution added rapidly to solutions containing the amount of ammonia in the various colorimetric determination will yield crystal-free mixtures.

Ammonium Sulphate Solution.—Folin and Denis recommend Kahlbaum's C. P. ammonium sulphate for the preparation of the standard. This is pure but

*Folin, Otto and Denis, W.: Nitrogen Determination by Direct Nesslerization, Jour. Biol. Chem., September, 1916, xxvi, 473 to 506.

must be dried in a desiccator for several days, or by heating it to about 110° C. for an hour before it is weighed out. The standard is made as follows: 4.716 grams of the salt is weighed out accurately and dissolved in one liter of 0.2 normal surphuric acid. The solution contains one milligram of nitrogen per c.c. and keeps very well. From this solution appropriate dilutions can be made.

Sodium Hydrate Solution.—100 grams of sodium hydrate are dissolved in one liter of water.

The Digestion Mixture.—300 c.c. of ordinary phosphoric acid "syrup" (containing about 83% H_3PO_4) are added to 100 c.c. of concentrated sulphuric acid. To this mixture 15 c.c. of a 10% solution of copper sulphate are added and the solution is filtered.

Pipettes and Glass Ware.—A long stem calibrated Oswald pipette, which delivers accurately 1 c.c. of water when drained for ten seconds against the side of a test tube and then blown clean, is required for measuring the urine. An ordinary 1 c.c. pipette, 200 or 250 c.c. volumetric flasks, micro-burners, hard glass test tubes (200 mm. × 13 mm.) and a Duboscq colorimeter, the zero point of which has been determined with care, complete the necessary equipment.

The Determination.—The urine is diluted so that 1 c.c. contains from 0.7 to 1.5 mg. nitrogen. In general, the following rules may be applied: Dilute urines having specific gravities of less than 1.018, five times; urines having specific gravites between 1.018 and 1.030, dilute ten times, and those whose specific gravities are above 1.030, dilute twenty times.

One c.c. of the diluted urine is measured by the Oswald pipette into a large test tube, and 1 c.c. of the phosphoric-sulphuric acid mixture is added. A fresh bit of granite is introduced to prevent bumping. The tube is then heated over a micro-burner, until nearly all the water has been driven off as indicated by the absence of foaming or by the appearance of denser sulphuric acid fumes within the test tube. The mouth of the test tube is then covered, and the heating continued with the flame regulated so that only a little of the acid fumes escape from the tube. In one-half to three minutes, counting from the time the test tube is closed, the digestion should be clear and blue, green, or light yellow in color. This color is due to the copper. The heating is continued for 30 to 60 seconds, but in no case should the time of heating be less than two minutes after the closure of the tube.

The test tube is then allowed to cool for two or three minutes and a little water is added and the contents rinsed into a 200 or 250 c.c. volumetric flask, using about 150 c.c. of water for the purpose.

The titratable acid content of 1 c.c. of the phosphoric-sulphuric acid mixture is now determined using 10% sodium hydrate with phenolphthalein as an indicator. The number of c.c. of alkali used in neutralizing the acid is then multiplied by 1.375. This figure represents the number of c.c. of alkali which must be added to the digestion mixture to make it neutral. Two c.c. in excess of this amount are added to make the solution definitely alkaline.

The standard solution is prepared as follows: Into another volumetric flask of the same capacity as that used in the case of the unknown, introduce 1 c.c. of the sulphuric-phosphoric mixture and 20 c.c. of an ammonium sulphate which is prepared from the standard stock solution by diluting it twenty times.

(The diluted solution contains 0.05 mg. nitrogen per c.c., and is a dilution quite appropriate as a standard for most nitrogen determination in blood and urine.) About 125 c.c. of water and the same amount of alkali that was used in the case of the unknown is then added and thoroughly mixed. Then to each flask 15 c.c. of the dilute Nessler reagent is added from a cylinder and the contents thoroughly mixed. The flasks are filled to volume and again mixed. A small part of the unknown is then poured out as an added precaution against incomplete mixing, and a portion of the remainder is filtered through a cotton plug or centrifuged. If the sediment thus obtained is mixed with a red deposit the Nesslerization is faulty and the fluid must be discarded. The liquid above the sediment must be clear and not "smoky." This fluid is to be used in comparison with the standard as follows:

Both cups of the colorimeter are filled with a portion of the standard and the colorimeter set at 20 mm. Adjust the cups to the same color intensity. When this is done, replace the fluid in one of the cups with the unknown and make one leisurely careful reading. This method of reading the colorimeter has been found to give the best results. As stated above the color of the standard is given by 0.05 mg. of nitrogen per 100 c.c. The calculation of the unknown is

$$\frac{20}{\text{reading of the unknown}} \times .05 = \text{mg. nitrogen in 1 c.c. of urine.}$$

THE DETERMINATION OF THE NONPROTEIN NITROGEN OF THE BLOOD.

Folin and Denis discovered that by using m.-phosphoric acid as a precipitant for the blood proteins, the nonprotein nitrogen of the blood can be determined by direct Nesslerization in a manner similar to that just described. The method is as follows:

Since *m.*-phosphoric acid (glacial phosphoric acid) is not stable in watery solutions; it should be prepared fresh from the sticks. The sticks weigh about 25 grams and will dissolve in 100 c.c. of cool water in an hour's time. The solution thus obtained can be used for two or three days. The other solutions required are the same as those used in the estimation of the total nitrogen in the urine.

Into 50 c.c. volumetric flask containing 20 c.c. of water, 5 c.c. of blood along with 3 c.c. of the 25% *m.*-phosphoric acid solution are introduced and mixed thoroughly. After allowing the mixture to stand for an hour the flask is filled to the mark, and the contents filtered through dry filter paper. If the first few drops are not water-clear, they must be discarded or put back upon the filter. If time is a factor it is not necessary to allow the mixture to stand an hour. In this case the volume is made up at once and the contents transferred to a larger flask, which is vigorously shaken for three to five minutes and filtered. Clear filtrates can be obtained in this way.

Ten c.c. of the clear filtrate, corresponding to 1 c.c. of blood, are placed in a large test tube and after the addition of 1 c.c. of the phosphoric-sulphuric acid mixture, the tube is heated over a micro-burner exactly as described for the urinary nitrogen. When the digestion is completed the contents of the test tube are rinsed into a 100 c.c. volumetric flask, using about 60 c.c. of water. The titrating value of the phosphoric-sulphuric acid mixture is obtained as

described above, and 1.375 times the amount plus 1 c.c. for alkalinity of 10% sodium hydrate is added. The flask is cooled and 10 c.c. of the dilute Nessler reagent is added, and the flask filled to the mark. Filter or centrifuge, if necessary, and the nitrogen is determined with the colorimeter, using as a standard the ammonium sulphate solution as described in the total urinary nitrogen method.

In cases where there is a very high nonprotein content, a standard of 1 mg. ammonia nitrogen may be required. In working with such blood the standard is best prepared as follows: The acid is diluted and the alkali is added to the flask which is to contain the standard, without adding the standard ammonia, until the color has been developed in the unknown. By inspection it can readily be told after a little practice how strong the standard should be, and the standard ammonia added accordingly.

THE DETERMINATION OF AMMONIA IN THE URINE BY DIRECT NESSLERIZATION.

The presence of creatinin in the amount that it is found in normal urine prevents the direct determination by Nesslerization of its ammonia. Folin and Denis have determined that if the amount of creatinin be reduced to a trace or even a large trace, direct Nesslerization is possible. This they do by the use of blood charcoal. The determination is carried through as follows:

Ten c.c. of urine, along with 1 c.c. of m.-phosphoric acid (25%), 9 c.c. of water, and 2 grams of blood charcoal (Merck's), are placed in a large test tube and vigorously shaken for a minute or so. The contents of the tube are filtered through dry filter paper, and 1 to 5 c.c. of the filtrate transferred to a 100 c.c. volumetric flask. About 70 c.c. of distilled water is then added and 15 c.c. of the dilute Nessler reagent previously described is quickly introduced. The contents of the flask is thoroughly mixed and made up to 100 c.c. A portion of the clear solution obtained is then compared in the colorimeter with the standard ammonia solution which has been treated with dilute Nessler reagent as previously described in the estimation of total nitrogen in the urine.

THE DETERMINATION OF THE UREA IN THE BLOOD AND THE URINE BY DIRECT NESSLERIZATION.

By using a 25% solution of *m.*-phosphoric acid as a precipitant of the urease materials used in the hydrolysis of urea, it is possible to determine the urea in the blood and urine by direct Nesslerization, as in the case of the methods previously described.

The urease can be prepared readily by rubbing up 5 grams of soy bean meal (The Soja bean meal sold as a food for diabetics can be used) with 5 c.c. of water; 400 c.c. of water and 100 c.c. of alcohol are then added. 10 to 15 c.c. of the suspension obtained are used in each determination. The preparation remains good for two days at room temperature.

The urea in the blood is determined as follows: 5 c.c. of blood and 10 c.c. of the suspension of soy bean meal are placed in a 50 c.c. volumetric flask, which is stoppered and allowed to stand for an hour. 25 c.c. of water along with 2 c.c. of the *m.*-phosphoric acid (25%) are then added and the flask made up to volume. The contents are thoroughly mixed and allowed to stand for 45 minutes or over night, after which they are filtered. A half gram of blood

charcoal is added to the filtrate, which is thoroughly mixed and again filtered. In the case of normal or approximately normal blood, transfer 10 c.c. of the last filtrate (corresponding to 1 c.c. of blood) to a 25 c.c. volumetric flask, and add 5 c.c. of the Nessler's reagent, make up to volume, mix, and compare at once in a colorimeter with a 0.25 mg. ammonia nitrogen solution (standard ammonia solution diluted one to four), Nesslerized in a 50 c.c. flask with 10 c.c. of Nessler's reagent. If less blood is used, a larger portion of the filtrate can be used for Nesslerization. The determination of the urea in the urine is not essentially different than that used in the determination of the urea in the blood.

One c.c. of urine is transferred with an Oswald Pipette to a 100 c.c. volumetric flask, along with 15 c.c. of the soy bean suspension. After allowing the mixture to stand for an hour, 25 c.c. of water and 1 c.c. of 25% *m.*-phosphoric acid are added and thoroughly mixed. One gram of Merck's blood charcoal is then introduced and about 25 c.c. of water. The contents are then thoroughly mixed, and made up to volume and filtered. From 5 to 20 c.c. of the clear filtrate are transferred to a 100 c.c. measuring flask (the amount should contain from 0.7 to 01.3 mg. nitrogen). Dilute with water to about 60 c.c. and Nesslerize in the usual way, and compare with a standard in the colorimeter. The standard should contain, in the majority of cases, 1 mg. nitrogen.

URINALYSIS IN RELATION TO RENAL LESIONS*

By J. R. Stark, M.D., Cincinnati, Ohio.

THE object of the work upon which this paper is based, was to discover what relation exists between urinalysis, the clinical diagnosis of nephritis, which is so frequently directly based upon urinary findings, and the anatomic changes in the kidneys. The material which served as the basis consisted of six hundred clinical histories in connection with which the term nephritis was used in the clinical diagnosis. All of the cases studied had gone to autopsy and therefore the anatomic renal material was complete. The clinical history, autopsy protocols, gross material, and microscopical preparations were therefore available for study.

It was very quickly discovered that the term nephritis, qualified or unqualified, was a loose one, and was often used with such apparent lack of interest or understudy that very frequently there was no clear clinical data in the histories to warrant it. For this reason the number of cases which were really useful for investigation reduced itself to 100, and to obtain even this number it was necessary to include those in which the clinical history included such terms as "albumin present," "blood and pus," or "albumin and casts." Such expressions are obviously inadequate ones upon which to base a discussion, and yet if clinical diagnoses are made upon such fragmentary bits of information, and if histories are to serve their function in the present or in the future, then these inadequacies, because they indicate clinical historical incompleteness, should have attention attracted to them.

*From the Mary M. Emery Department of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.

After a preliminary survey of these 100 cases they were divided into three groups, as follows:

I. Those in which the clinical and anatomic diagnosis did not agree, 26.

II. Those in which the clinical and anatomic diagnosis agreed, 36.

III. Those in which there was no clinical diagnosis, but in which the clinical data indicated some renal abnormality, 37.

GROUP I.

In this group there was, as already noted, a discrepancy between the clinical conclusions and the anatomic findings. For instance, nine cases were clinically diagnosed as chronic diffuse nephritis, whereas at the postmortem table they proved to be acute. Seven were just the reverse, clinically they were acute nephritis; anatomically, chronic. In five cases the kidneys were tuberculous. The clinical diagnosis in two of these was acute nephritis; in two, chronic nephritis; and in one, cystitis. There were five other cases belonging in this group. One was diagnosed clinically as tuberculous nephritis and proved to be carcinoma of the kidney; two were clinically acute parenchymatous and anatomically diagnosed as "nephritis" and proved to be chronic diffuse nephritis.

The facts mentioned above are tabulated as follows:

TABLE I.

CLINICAL DIAGNOSIS.	ANATOMIC DIAGNOSIS.	NO. CASES.
Chronic diffuse nephritis	Acute nephritis	9
Acute nephritis	Chronic diffuse nephritis	7
Acute nephritis	Tuberculous nephritis	2
Chronic nephritis	Tuberculous nephritis	2
Cystitis	Tuberculous nephritis	1
Tuberculous nephritis	Carcinoma	1
Acute parenchymatous nephritis	Chronic diffuse nephritis	2
"Nephritis"	Chronic diffuse nephritis	2
Total		26

The five tuberculous kidneys are probably the most interesting and the urinalysis and clinical symptoms are worthy of especial attention. In the clinical cystitis case, there was a history of frequent and painful urination with a urinalysis of "S.g. 1010, albumin trace, few red blood cells, no pus or casts" and no other tuberculous lesion was found in the body. The two chronic nephritides showed urines with the following analyses respectively—S.g. 1024-1002, albumin trace, no cells or casts in either. The two acute nephritis urinalyses showed respectively S.g. 1020—not given, albumin neg.—a trace, few granular and hyaline casts, no blood—few red blood cells, and no casts. These last four cases had definite pulmonary lesions but no history of any urinary disturbance. The contrast between the two supposedly acute nephritic cases are typical examples of what one may expect to learn from urinalyses, as will be shown more fully in the course of this paper. Not only are they not the analyses of acute nephritis, but they mislead one as to the true condition existent.

The typical analyses referred to, which one ordinarily regards as definite, are, briefly mentioned: Urine in acute nephritis is decreased in amount, or com-

pletely suppressed, S.g. 1025 to 1028, sediment large, and albumin in large amounts, as high as 8.5 per cent. Microscopically there are usually red and white blood cells in variable amounts, epithelial cells, hyaline, blood and epithelial casts. The urine in the chronic diffuse nephritides with more of the interstitial change, shows an increased output, S.g. 1005 to 1012, very scanty sediment, albumin as a trace or completely absent, solids decreased in amount. Microscopically there is often a shower of red blood cells, fine and coarse granular or hyaline casts. A kidney of the parenchymatous variety of the chronic diffuse nephritis type, is expected to excrete a decreased amount of urine with a S.g. of 1020 to 1040, a heavy sediment, albumin present in large amounts $\frac{2}{5}\%$ by weight. Microscopically there are hyaline epithelial, fine and coarse granular, and fatty casts. Leucocytes are abundant with occasional red blood and epithelial cells.

The above simple analyses only take into consideration those factors which are at every one's disposal and are more or less routine procedures in an institution.

In order to make this group more complete the urinalyses in the remainder of the cases are given as completely as they were in the history.

TABLE II.

URINALYSES OF CASES CLINICALLY DIAGNOSED CHRONIC AND ANATOMICALLY ACUTE.

S.g.	Albumen.	Casts.	Cells.
?*	Trace	None	Reds and whites
?	"	?	?
1012	?	Very many cellular	Pus and reds
1022	+++	Granular	None
1020	Neg.	Hyalin and Granular	?
1018	"	Neg.	Reds
1020	+	?	?
1016	Neg.	Neg.	Neg.
1012	+	Granular	No Reds

?*Not given in the history.

TABLE III.

URINALYSES OF CASES CLINICALLY ACUTE AND ANATOMICALLY CHRONIC.

S.G.	Albumen.	Casts.	Cells.
1022	+	Large amt. granular	?
?	++	?	?
?	++	Abundant granular Epithelial, hyaline	pus, no reds
1040*	+	Granular	?
1032	++	?	?
1040	+	Granular, hyaline	?
1006	++	Granular, hyaline	?

*Sugar present in urine.

The above analyses, incomplete as they are, are still sufficient to show how little one may depend upon an analysis in nephritic lesions.

GROUP II.

In thirty-seven cases the clinical and pathological diagnoses agreed. The next question which arose concerning this group was, in how many cases where

the correct diagnosis was made did the analysis given correspond even fairly accurately to the composite description herein given? Only fourteen out of these thirty-seven cases showed the typical analysis which could warrant its being an aid in the diagnosis.

TABLE IV.

No. of clinical and anatomical chronic interstitial nephritis cases with typical urinalysis	5
No. of clinical and anatomical acute nephritis cases with typical urinalysis.	6
No. of clinical and anatomical chronic parenchymatous nephritis cases with typical urinalysis	1
No. of clinical and anatomical pyonephrosis cases with typical urinalysis..	2

In the other cases the chief factor which was at variance was the specific gravity. Where given, it was almost invariably too high by quite a wide margin. In chronic diffuse nephritides the albumen was often marked three plus (+++) while in five acute conditions the only notice that was taken of albumin was the laconic expression of "present." Casts were too variable a quantity to deserve much mention. Coarse granular casts were found in the acute nephritides while in others no casts were found. The chronic interstitial varieties in most instances contained all varieties in considerable amounts.

In only a few of the sixty-three cases was albumin absent and yet its presence in large amounts was not an indication of the amount of renal damage. The converse proved to be equally correct.

As an example of the conditions frequently encountered, the following case covers a number of the points: In a fulminating case of lobar pneumonia the urine showed "S.g. 1020, albumin negative, a few granular and hyaline casts, and no abnormal cells." The clinical diagnosis was chronic diffuse nephritis, which corresponds very closely to the typical urinary picture except for the high specific gravity. Postmortem these kidneys proved to be involved in an extreme grade of acute nephritis, with no chronic change as one might expect from the clinical condition. This case is not an exceptional one but is a typical example of the discrepancies which one meets daily.

The high specific gravity in all cases would tend to mislead one did he not consider that the kidneys are only one exit for the elimination of water. In fevers with profuse sweating, in cases in which there is diarrhea, and in cases in which but little water is ingested, it is to be expected that the specific gravity would rise above that usually described in chronic nephritides. Further, the total solid excretion probably bears little if any relation to the water output, for in the functional renal test we often get a high percentage of phenolsulphonphthalein with a small amount of fluid excretion, while in diabetes with polyuria the specific gravity is high.

Also leucocytes or "pus" are not found where most expected. In five cases showing infarction, acute interstitial nephritis or "surgical kidneys" the urine was free from white blood cells, while in two others where the report was "quantities of pus" and the clinical diagnosis cystitis, the postmortem examination revealed pyonephritis.

It must not be considered that these comparisons are from picked or unusual cases. They represent typical clinical reports with their postmortem veri-

fications or contradictions. Of course, sixty-three cases may hardly be sufficient upon which to base a radical report, yet if one realizes that in at least five hundred and fifty out of the six hundred cases studied there was some nephritic lesion, and that of this number only sixty-three cases having both a urinalysis and a clinical diagnosis could be obtained, then it seems apparent that the clinicians also lay very little stress upon urinalysis as a diagnostic measure. So we are led to the conclusion that a urinary examination is of as little value, in itself, as a leucocyte count, a Wassermann, a gastric analysis, or other laboratory procedures without other data, and that in the differential diagnosis of kidney lesions the urinary picture is of practically no importance.

TECHNIC FOR STAINING BLOOD, ESPECIALLY FOR PLASMODIA

By Wm. Krauss, M.D., Memphis, Tenn.

STAINING technic. Dissolve 25 centigrams eosin, w.g. in 25 c.c. fresh distilled water. Label: No. 1. Dissolve 25 centigrams azur II in 25 c.c. fresh distilled water. Label: No. 2. Provide also chemically pure absolute methyl alcohol, fresh distilled water, anilin oil, each, a sufficient quantity; a 10 c.c. graduate; a Petri dish or special staining dish.

Note: The stains are best kept in glass stoppered "balsam bottles." These have "outside closure" and admit dropping pipets easily. "Ice house" distilled water is rarely fit for this purpose. It is well to have the special staining dish (Sargent) and the slide attachment for the centrifuge. (B. & L. O. Co.)

Proceed as follows: Flood the slide with the methyl alcohol. Measure 5 c.c. distilled water, add a drop of anilin oil, shake; add two drops No. 1, shake; add four drops No. 2, shake; pour into dish. After at least one minute's fixation in the alcohol, put the slides face down into the stain. The adhering alcohol may remain. Leave undisturbed for ten minutes. Remove slides, dry on centrifuge, examine under oil.

N. B.—Fungi may develop in the staining solutions and absorb stain. This necessarily changes the proportions; also, the droppers may be of unequal size. The proportion is right if the finished mixture is quite blue when seen in glass dish over white paper. The leucocytes must show very deeply stained, otherwise the parasites remain unstained. Never use the same dropper in both solutions, as they precipitate each other. To remove smudges and precipitates from the stained smear, gently wipe off with a pledget of cotton dripping wet with aceton, going over the slide *only once*.

A CONVENIENT ELECTRICAL SUPPLY SYSTEM FOR A PHYSIOLOGY LABORATORY*

By Clayton McPeek, M.D., C. I. Reed, A.B., and Frank Beck,
Columbus, Ohio.

A CONVENIENT system of electrical supply is of considerable importance in a physiological or pharmacological laboratory. In most schools, the dry cell is still employed as a means of supply for use in experimental work. While this affords an independent supply for each student, or each group of students using the same set of apparatus, there are the disadvantages of constant expense in the purchase of new cells, time loss on the part of the student in securing and connecting new batteries, the clumsiness and inconvenience of the apparatus when the batteries are connected, and the constantly varying supply of current from this source.

On account of these disadvantages a new system has been installed in this laboratory, which, after several months of trial in class work has proved so satisfactory that it has seemed desirable that it be brought to the attention of others who may care to profit by its use.

The apparatus consists of a motor generator charging set, manufactured by the Robbins-Myers Co., of Springfield, O., which may be operated by either an alternating or direct current at either 110 or 220 volts. This apparatus generates 10 amperes at 8 volts D. C., charging a three cell storage battery of the "Ever-ready" type. This battery is of 6 volt, 80 ampere-hour capacity and is mounted on a shelf under the switchboard. On the switchboard are placed fused switches, jacks, fuses and parallel and series bar connections. The switchboard is made of a 1-inch marble slab, 12 inches by 24 inches, supported by iron brackets so as to leave a 5-inch space between it and the wall for making final connections after it is in place. The connections may be traced out by reference to the diagram in Fig. 1.

The 110 volt supply enters through the fused switch S, then passing to the motor end of the generator through the rheostat R, by means of which the speed and proper charging rate for the battery may be regulated. The current from the generator, at 7 to 8 volts, passes through the fused switch C.

When the battery is to be charged, both switches are closed. The current will flow from the positive (+) terminal of the switch C to the positive terminal 6, through the fuse and jack G to one cell of the battery; from the negative (—) pole of the cell to terminal 5, through the series bar B to terminal 4 and through jack F to the middle cell of the battery to terminal 3 and again through the series bar B to terminal 2 thence through the fuse to the jack E, and the remaining cell of the battery to terminal 1, thence to the negative side of switch C.

When the charge is completed, switch C should be opened first, otherwise the battery will discharge into the generator, since all jacks, terminals, fuses and switches are back-connected on the switchboard.

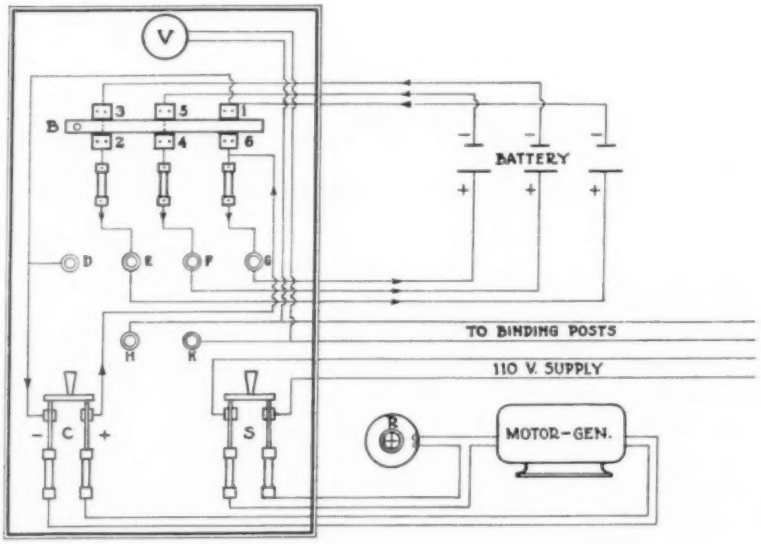
Jacks D, E, F, G, H and K are made of brass of 5/8 inch diameter, turned

*From the Laboratory of Physiology of the College of Physiology of the Ohio State University.

down in a lathe, with a head, threaded to ½ inch and drilled out having an opening of about ¼ inch in diameter. These are placed in holes drilled through the switchboard and held in place by nuts on the reverse side of the board. Under the nuts are brass clips for the connections with the circuit wires.

Terminals 1, 2, 3, 4, 5 and 6 are of spring brass 1-16 inch thick and 1 inch wide, bent into right angles and fastened to the board with brass machine screws which pass through the marble and support brass clips to which wires are soldered for the rear connections of the terminals. The two series of the terminals are ⅝ inch apart; into this space is inserted the series bar, used in charging or discharging the battery.

The series bar is of hard wood ½ inch thick with brass contact plates 1-16 inch thick, mounted on each side, corresponding to the positions of the ter-



minals. Copper rivets form connections between 2 and 3 and also between 4 and 5. Contacts 1 and 6 are not so connected. A ¼ inch hole in one end of the bar fits onto a brass stud of corresponding size, mounted on the board, so that there can be no error in inserting the bar.

The parallel bar is also of hard wood, with contacts of brass mounted on each side so as to connect in parallel, terminals 3, 5 and 1 and also 2, 4 and 6. When the battery is not in use, the parallel bar is inserted. This permits of an "evening-up" between the cells of the battery so that all may be kept at, approximately, the same voltage. This bar may also be used when only two volts are required on the circuit, so bringing an equal load upon all the cells.

With this arrangement, three voltages may be supplied to the circuit H-K which leads to the binding posts on the desks in the laboratory. The connections between the jacks and the laboratory circuit are made by means of two cables about 18 inches long whose wires, passing through wooden handles at each end, are soldered onto brass plugs attached to the end of the handle. The plugs are ¼ inch in diameter and slotted so as to form good connections when inserted into the jacks.

If one cable connects D to H and the other connects E to K, with the series bar in place, two volts are discharged. With D and H still connected, if K is inserted in F, 4 volts will be obtained. With the connections between D and H, and G and K, 6 volts will be discharged. If all the cells are not needed, and it is desirable to employ 4 volts, the load may be shifted by connecting E to H and G to K.

In charging the series bar is always used. Periodic readings of the specific gravity of the fluid in the cells are taken by means of a special hydrometer designed for the purpose. Whenever the gravity falls as low as 1.150, the battery should be charged until it reads 1.270. A fall in the voltage discharge as indicated by the readings of the voltmeter, V, which is tapped onto the circuit, indicates recharging. Each cell usually registers 2.1 volts. Charging can usually be done outside of the regular laboratory periods. Unless it is unavoidable, the cells should not be charged while the students are using the current for the generator will produce variations in the strength of the current that sometimes seriously disturb the experiments in progress.

Should it be desirable to test the voltage of the cells separately, the voltmeter may be detached from its connections with the laboratory circuit and applied to each cell.

The laboratory circuit is conducted by wires led through conduits under the floor and brought up to binding posts mounted on wooden blocks between the ends of the desks so that the students at each desk may have free use of one set of binding posts. A circuit from an electric time clock is also led through the same conduit and brought up to a separate set of posts mounted on the blocks between the desks.

Incidentally, it may be mentioned that pipes for gas and compressed air are brought up from the floor between the desks and fitted with two way outlets, so that each desk is supplied with gas, air, electricity and time current, independently. This arrangement removes the necessity of overhead wires or piping, which are both inconvenient and unsightly.

NUMBER OF KEYS IN CIRCUIT.	DISCHARGE VOLTAGE AVAILABLE.	
	2.1	4.1
1	2.0	3.7
2	1.9	3.3
3	1.8	3.0
4	1.7	2.75
5	1.6	2.62
6	1.6	2.5
7	1.55	2.42
8	1.5	2.4
9	1.45	2.3
10	1.42	2.21
11	1.4	2.12
12	1.32	2.05
13	1.3	2.0
14	1.28	1.9
15	1.2	1.85
16	1.1	1.78
17	1.02	1.63
18	.9	1.54
19	.8	1.43
20	.76	1.2

In order to determine the efficiency of the system, a series of tests were made, in which the students were instructed to connect the inductoria with the binding posts at the desks, with a simple key in circuit. A voltmeter was connected with one pair of binding posts and readings were taken as the keys were closed, one at a time. Tests were made with line supplies of 2 and 4 volts. The results are shown in the preceding table. The first column shows the number of keys closed; the second shows the voltmeter readings with a line discharge of 2.1 volts; the third column shows the voltage with a battery discharge of 4.1 volts.

It will be apparent from this table that there is considerable variation in the line voltage when a large number of inductoria are in use at the same time. This disadvantage may be obviated if the battery is so placed in the laboratory that several feed lines may be leading off from it to the desks, thus placing only a few desks on one line. On account of the arrangement of this laboratory, it was found necessary to place the battery in a small room from which only one supply line could conveniently be led into the laboratory, hence all of the 32 desks are tapped onto one line. However, since but few of the students are using the current at the same time, the system is still more satisfactory, even under these conditions, than the old method of obtaining current from dry cells.

At first, there was installed a generator without the storage battery, which supplied current as needed. It was found, however, that this caused a vibration in the current in the primary coils of the inductoria sufficient to induce a very weak faradic current in the secondary coil. This interfered seriously with the efforts of the student to obtain satisfactory results on simple make and break stimulations. This also constitutes a disturbing factor if the battery is charged when the students are using the current. A series wound motor-generator can be used with more satisfaction than a shunt wound one.

Aside from this one disadvantage of variability in voltage, which does not become a disturbing factor under most conditions of laboratory experimentation, this system presents a number of advantages. The entire first cost of installation did not exceed $125.00, and the expense of current and upkeep is very small. There is also a great saving of time to both the student and the instructor. There is a fairly constant supply at all times and the outlets are so distributed that the student can move his apparatus to any part of the laboratory and still be within easy reach of any set of binding posts. There is also a minimum amount of time required on the part of the student with relation to accessory apparatus, no part of the system requiring a great deal of attention in order to keep it in good condition and ready for use.

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Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
Ann Arbor, Mich.

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EDITORIALS

Tuberculosis

THERE is a demand on the part of the intelligent laity that the crusade for the restriction of tuberculosis be pushed with all the intelligence and enthusiasm that the medical profession can command. The verdict of science is that tuberculosis is a preventable disease; one which we can reasonably hope to wholly eradicate in time from the list of diseases that afflict the race. The intelligent laity accepts this verdict and demands that the medical profession follow the way pointed out by science. Thirty years ago a sanitarium devoted to the care of tuberculosis patients was a rare thing. There were not many medical men at that time who had the visions which Trudeau saw in the forests about Saranac. Now, there are hundreds of sanitoria devoted to this disease. Nearly every state has one or more such institutions and municipalities are doing even better. Forty years ago the best physician was not sure that anyone ever recovered monary tuberculosis, his patient must show marked emaciation, a chronic cough, profuse expectoration, and an afternoon elevation of temperature. Practically all who reached this stage died and the conclusion generally reached by the from this disease. Before he could make an unquestionable diagnosis of pul-

best physicians was that pulmonary tuberculosis terminated fatally in one hundred per cent. This belief was quite universally held by the laity and the physician, who had a heart like other men, postponed announcement of his diagnosis, he equivocated, he temporized, and often he lied directly. At that time and under the conditions then existent, this was to his credit rather than otherwise, but he got into a habit which many older men have never been able to cast aside and more unfortunately still, many younger ones have acquired. In the sixties Villemin demonstrated experimentally that tuberculosis is an infectious and transmissible disease, but his work threw no light on the diagnosis, and while the up-to-date physician might take some precautions against the spread of the disease, he gained in nothing else. In the eighties Koch identified, isolated and grew in pure cultures, the infective agent, the existence of which in tuberculous sputum, Villemin had demonstrated. For a time after Koch's discovery the identification of the specific bacillus was deemed essential to a positive diagnosis. This was a step in advance, but a short one. "If we wait until tubercle bacilli have appeared in the sputum, in the majority of instances we have waited too long. At present with marked advance in the interpretation of physical signs, by a close study of the clinical fluctuations in temperature, especially as influenced by exercise, by x-ray studies and by means of the different tuberculin tests, the disease can in most instances be recognized while it is yet in its incipiency and while it is highly amenable to hygienic treatment. Hundreds, and in some sections, thousands of people are now going to the physician to find out whether they have the infection. Forty years ago it was a cruel thing to pronounce a diagnosis of tuberculosis even when its existence in an advanced stage was plainly evident. Today failure to recognize this disease in its earliest stages shows ignorance on the part of the physician and may result in irretrievable injury to the patient.

The wide-awake, conscientious physician is on the lookout for every scientific advance in our knowledge of tuberculosis. Doctor Gerald B. Webb, of Colorado Springs, has consented to become a member of our editorial staff and will devote his energies to the subject of tuberculosis.

—V. C. V.

The Journal of Laboratory and Clinical Medicine

VOL. II. ST. LOUIS, DECEMBER, 1916 No. 3.

ORIGINAL ARTICLES

AN EXPERIMENTAL INVESTIGATION OF CERTAIN PHENOMENA RELATING TO THE ACTION OF DRUGS ON THE RATE OF OXYGEN CONSUMPTION IN THE ANIMAL BODY*

BY D. E. JACKSON, PH.D., M.D., ST. LOUIS, MO.

A SIMPLE, cheap form of apparatus for recording the relative rates of oxygen consumption by an anesthetized animal is shown in Fig. 1. This apparatus is practically identical with that described in a recent article[1] on the employment of closed ether anesthesia for ordinary laboratory experiments. In fact I have used the same apparatus often for both purposes at the same time. For a detailed description of the construction of the apparatus (which costs but little and is intended primarily for *student experiments*) I must refer those interested to the former article. It will be seen from the illustration that a round disc of very thin aluminum (or pasteboard) has been attached (with mucilage) to the center of the upper surface of the rubber bath cap which covers the large pan into and out of which the animal breathes. From the aluminum disc a thread passes up over a pulley and thence to a second pulley placed above the frog heart lever which writes on the drum. The thread is attached to this lever by a bull dog clamp which serves also as a counterpoising weight to counterbalance the two or three bull dogs which usually have to be placed on top of the aluminum disc to cause it to descend readily as the animal inspires. The method by which a record of the oxygen consumption is obtained on the drum is shown in the figure. This record has a saw-tooth-like appearance. The narrow, almost perpendicular lines (which should normally all be of the same length) are made by the writing lever when oxygen is run into the pan. The drum should be running at a very slow speed (or even stopped for the few seconds required for the oxygen to run into the pan) when these lines are made. The broader, ascending curves represent two things, first the amount of air which actually passes into

*From the Department of Pharmacology of Washington University Medical School.

and out of the lungs at each inspiration and expiration. A contraction or dilatation of the bronchioles will, of course, show its effects immediately on this curve. And second, the curve forms a record of the relative rate of oxygen consumption by the animal. Both of these points are often of great interest in studying the action of drugs. It will be noted that the oxygen is run into the pan only at intervals and the signal for its admission is the arrival of the broad ascending curve to the same height as that which the previous saw-tooth had. Just enough oxygen is then quickly injected to bring the writing lever down to the former low point. Thus the height of each saw tooth should be practically the same.

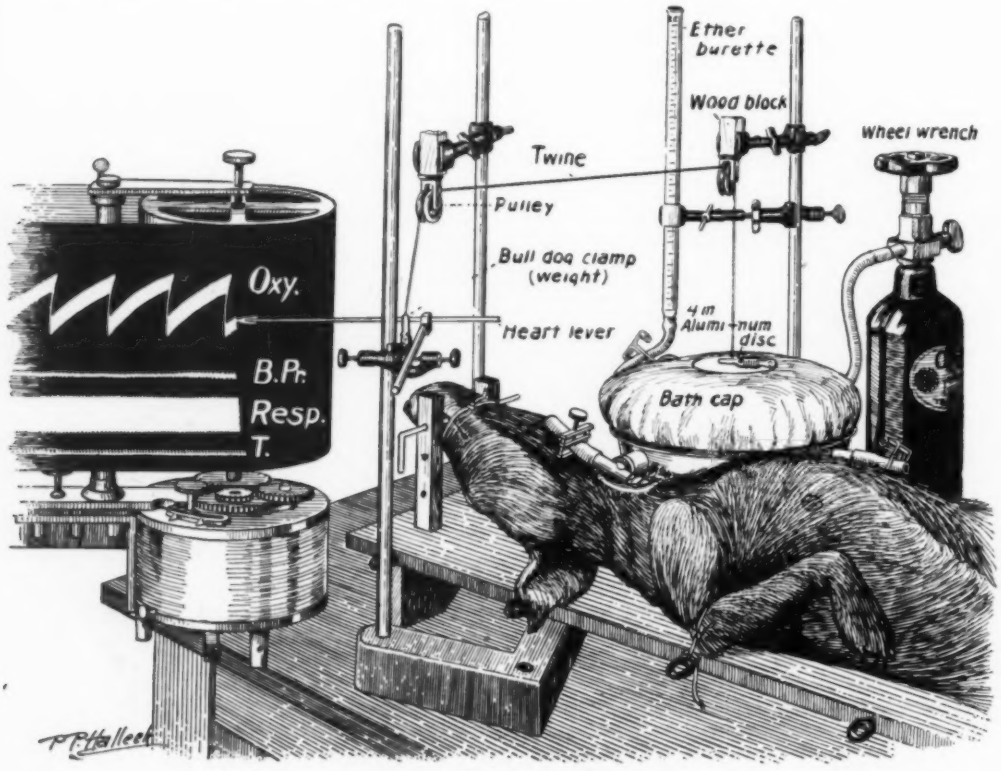

Fig. 1.

This is a relative matter which can be set arbitrarily to suit the operator for any given experiment or for any sized dog.

The distance between the descending lines marks the relative amount of oxygen which the animal consumed during that period of time (recorded in seconds, etc., on the drum). For example if in one three minute interval the animal consumed a given amount of oxygen and the first saw-tooth was made to correspond with this, but in the second three minute interval the dog consumed only half as much oxygen then the distance covered by the second saw-tooth will be twice as great as that covered by the first.

Special emphasis must be laid on the fact that the animal is excreting carbon dioxide all the time and this carbon dioxide is being continually absorbed by the strong sodium hydrate solution in the bottom of the large pan. The rate

of this absorption varies but little and can not interfere with the correct interpretation of large variations in the rate of oxygen consumption. Small variations in this rate which may be quickly compensated for by the animal may apparently often be somewhat obscured, especially when observations extend over only a brief period of time. But if a longer period is used then any sudden large excretion of carbon dioxide will be absorbed and the relative amount of oxygen consumed in the period will be shown on the record. This equalizing influence of longer periods of time seems to hold true for temperature (and perhaps for water and ether vapor variations) also.

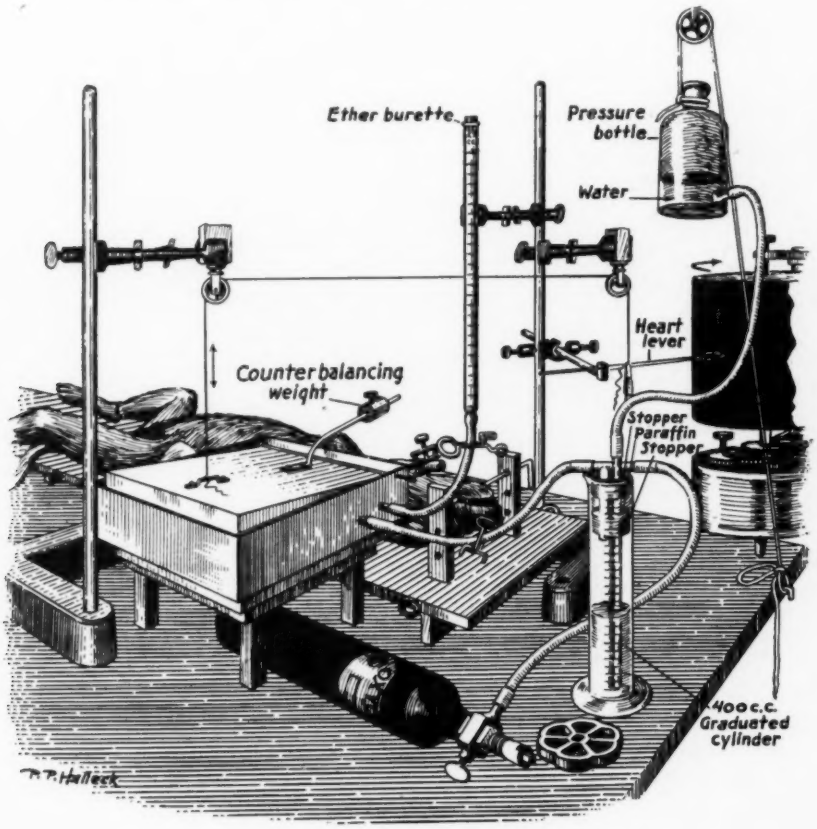

Fig. 2.

A better but somewhat more complicated form of the apparatus is shown in Fig. 2. Here a large, shallow, square pan is used as an absorption chamber. The details of the construction of this pan are shown in Figs. 3 and 4. A breathing pan made of exceedingly thin aluminum or brass (the latter is preferable) is hinged at the back and can dip up and down in a trough of water which fills the space between the outer wall of the large pan and a second inner wall which is soldered at the bottom to the floor of the large outer pan. This inner wall forms a second pan within the large outer one. Strong (not saturated) sodium hydrate solution is placed in the inner pan to the depth of about one inch. The breathing pan then is lowered and dips into the trough of water all

around thus forming an air-tight chamber into and out of which the animal breathes through the large spout at the back. The tracheal cannula (side tube) is passed through a large cork which is inserted into the spout. The breathing pan is carefully balanced on the hinges by an adjustable counterbalancing weight. The apparatus is thus made very sensitive and the mechanical hindrance to the breathing of the animal is reduced to a minimum. As shown in Fig. 2 a twine string is attached to the top of the breathing pan. This string

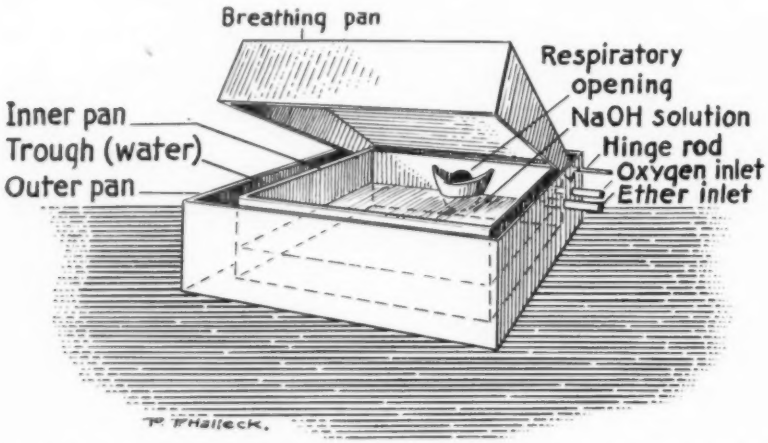

Fig. 3.—Interior view of the apparatus shown in Fig. 2. The "hinge rod" can be pulled out endwise to free the "breathing pan" which can then be entirely removed.

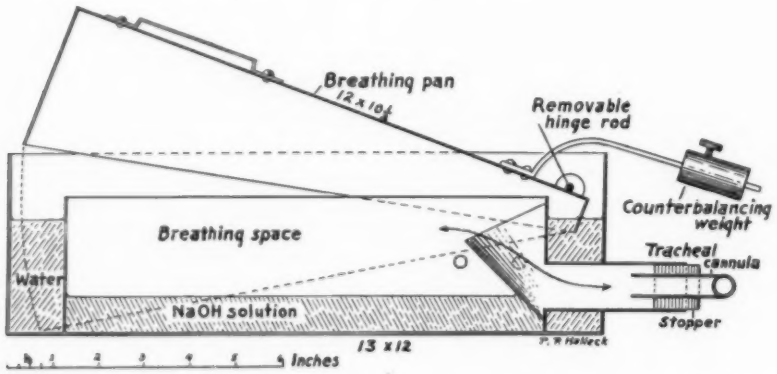

Fig. 4.—A longitudinal cross-section through the middle of the apparatus showing details of construction.

passes over two pulleys and finally is clamped with a bull dog forceps in the proper position to the long arm of a heart lever which writes on the drum.

An oxygen measuring device is also shown in Fig. 2. Oxygen under pressure is injected from a tank into the graduated cylinder through a T-tube the side tube of which passes through the two corks and a layer of paraffine which seals the upper end of the cylinder down to the graduation marks. The remaining straight end of the T-tube connects to a rubber tube which is ordinarily closed by a spring clip but which when open conducts the oxygen into the breathing pan. A pressure bottle containing water is suspended above the apparatus. A

tube leads from the pressure bottle down to the graduated cylinder where it is connected with a small glass tube which passes through the corks and paraffine and reaches down to the bottom of the graduated cylinder. Within the cylinder about 100 to 300 cubic centimeters of water is placed. If the oxygen tank be opened a little, oxygen will be forced at once into the top of the graduated cylinder. The water in the lower part of the cylinder will be forced up into the pressure bottle. Thus the cylinder can be quickly filled with oxygen. The tank valve is then closed and the charge of oxygen remains stationary in the cylinder waiting to be run into the breathing pan. This is done by simply opening the spring clip in the tube between the cylinder and the pan. The water in the pressure bottle will at once run down into the cylinder and force the oxygen over into the pan which will rapidly rise. On the drum this would be indicated by the abrupt descending limb of the saw-tooth record. The amount of oxygen thus run into the pan at each emptying of the cylinder can be only approximately measured on the cylinder because the glass tube inside passes down through the cylinder, and the water remaining in the tubes, etc., still further changes the cylinder readings as does also the pressure variations. The cylinder therefore is used only for approximate estimations. The exact measurement of the amount of oxygen in each charge (which is constant and is determined by the amount of *transferable water* placed in the cylinder) is obtained by filling the cylinder with oxygen (or air), then disconnecting the inlet rubber tube from the pan and collecting from this tube under water in a second graduated cylinder the oxygen (or air) as it is discharged from the first cylinder on opening the spring clip. By the use of this automatic measuring device oxygen can be quickly and accurately run into the breathing pan and always under the same pressure. And in making the records the greatest difficulty which one experiences is in determining just at what moment the writing lever has reached its former high point on the drum. This is best done by placing the writing pointer of a stationary tambour or signal magnet in such a manner that as the drum turns this pointer will mark a line on the surface of the drum just at the level of the apex of each saw-tooth in the record. This stationary writing point should be placed just in front (to the right on most drums) of the recording heart lever. By watching the ascent of the lever the observer can then come very close to determining the correct moment to inject a new charge of oxygen. (I unfortunately did not do this until after I had used the method for some time.) If there is an error in the time selected for the injection, however, this will be detected at once when the oxygen is run in for the lever will not descend to its former low-level line, but will either be below (injection made too soon) or too high (injection made too late). Thus a double check on each injection can be at once obtained. An obscuring influence which is sometimes present is a marked change in the depth of respiration of the animal. With drugs which cause these changes the high point on the record is sometimes difficult to determine. With strychnine, e.g., this point is hard to make out at the top of the record when convulsions come on but with a little experience one can soon make a very close approximation. Since each saw-tooth should cover only one to three minutes and the amount injected at one time can be checked up immediately with the following saw-

tooth, small variations due to respiratory disturbances can only cause errors for a brief time.

The form of anesthesia used with this apparatus is a matter of some concern. For ordinary purposes when dogs are used a small dose of chloretone is advisable. In addition to this a little ether, perhaps two or three cubic centi-

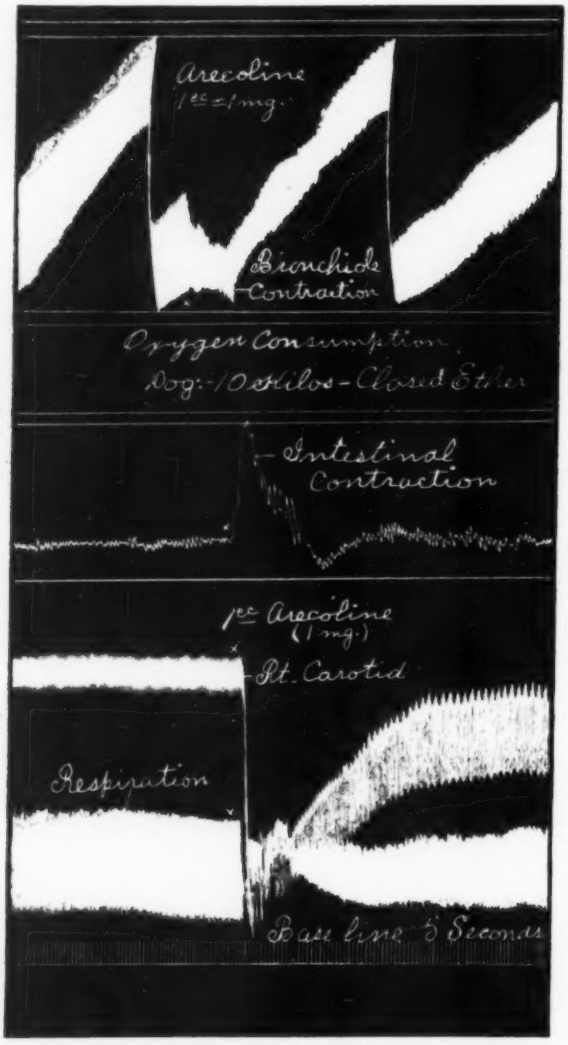

Fig. 5.

meters, may be injected (from a burette) into the pan. A perfectly even regular anesthesia should then be maintained. Ether alone may be used but there is a slight source of error in this since when the amount of oxygen in the pan is small (pan down) the concentration of the ether vapor breathed may be greater than when more oxygen is present (pan up). This might be sufficient to obscure some of the readings. In a few experiments I have used the apparatus for spinal

dogs. To do this I have used a special piece of apparatus[2] which I inserted into the chest (to hold the walls open and rigid) and then negative (interrupted) pressure was used to aspirate the chest at regular intervals. Artificial respiration was thus carried on after the animal's brain was destroyed. Perhaps this method might be used to investigate a number of problems concerning metabolism in animals in which the central nervous system plays only a minor role, or perhaps takes no part at all if both brain and cord are destroyed.

There are a few sources of error which may be present in this method. As indicated in the article referred to above[1] some carbon dioxide accumulation

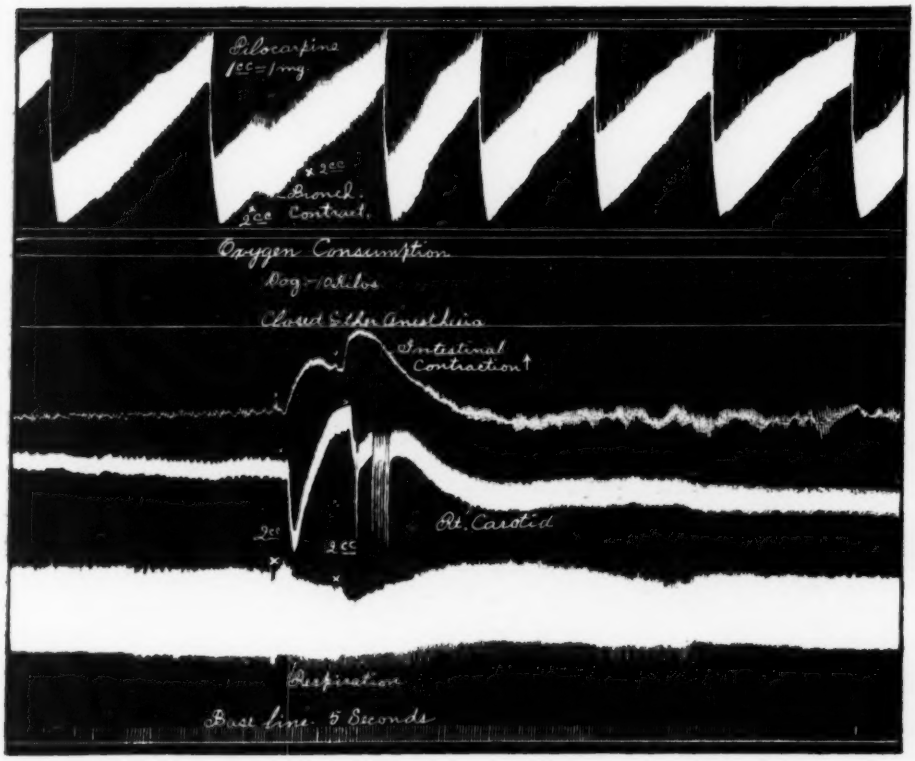

Fig. 6.

may occur in the breathing pan. With a strong fresh solution of sodium hydrate (*which must always be cold when it is used*) this does not appear to cause any practical disturbance provided the absorbing surface of the hydrate solution is great enough (large pan). A cold solution in the pan, cold water in the trough (Fig. 2) and the cold metallic walls of the pan (or the thin rubber bag in the apparatus shown in Fig. 1) serve to counteract the rise in temperature which the respired air might otherwise undergo. The careful counterbalancing of the breathing pan (or of the weight of the aluminum disc on the bath cap) together with the large, free, open spout through which the animal breathes serves to reduce to a minimum any mechanical obstruction to the respiration.

There are some interesting and obscuring influences which certain drugs

develop within the animal itself, however, and while these may sometimes interfere with the use of this method for determining the rate of oxygen consumption, the method, on the other hand may be used to great advantage for studying these very phenomena.

Among these drug reactions should be mentioned first of all perhaps the sudden increase in the rate of carbon dioxide excretion which a rapid increase in the rate and depth of respiration may bring about. In a brief period of time

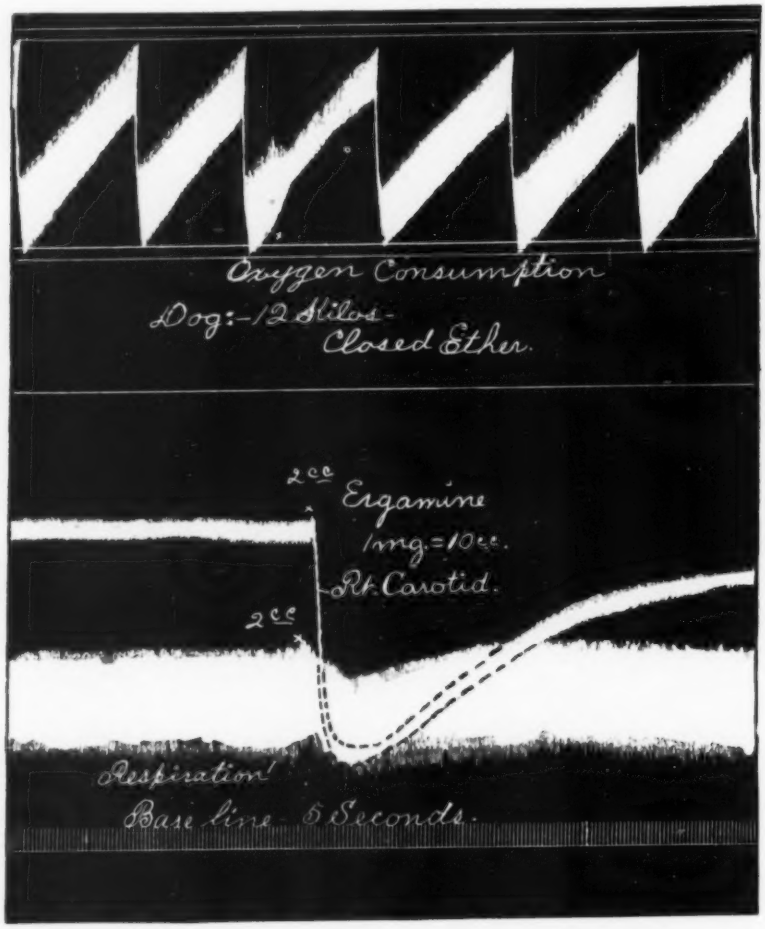

Fig. 7.

this excess of CO_2 will affect the record but after a time the sodium hydrate solution appears to catch up in its rate of absorption. Again if the animal suddenly begins to breathe deeply the expired air may be warmer (more expanded) than before and an excess of watery vapor may be thrown off. A large pan and plenty of cold solution, etc., will overcome this also when the respiration of the animal returns to normal. Again the injection of a drug which is wholly or partly excreted by the lungs (e.g., sodium sulphide, after which small amounts of H_2S escape in the breath) as a gas or vapor may affect the record. The in-

jection of a small amount (1/2 c.c.) of ether from the burette into the pan may affect the record at once, especially if the temperature of the room or pan is moderately high. Presumably this would also be noticed after the intravenous injection of a solution of ether, although I have not yet tried out the experiment.

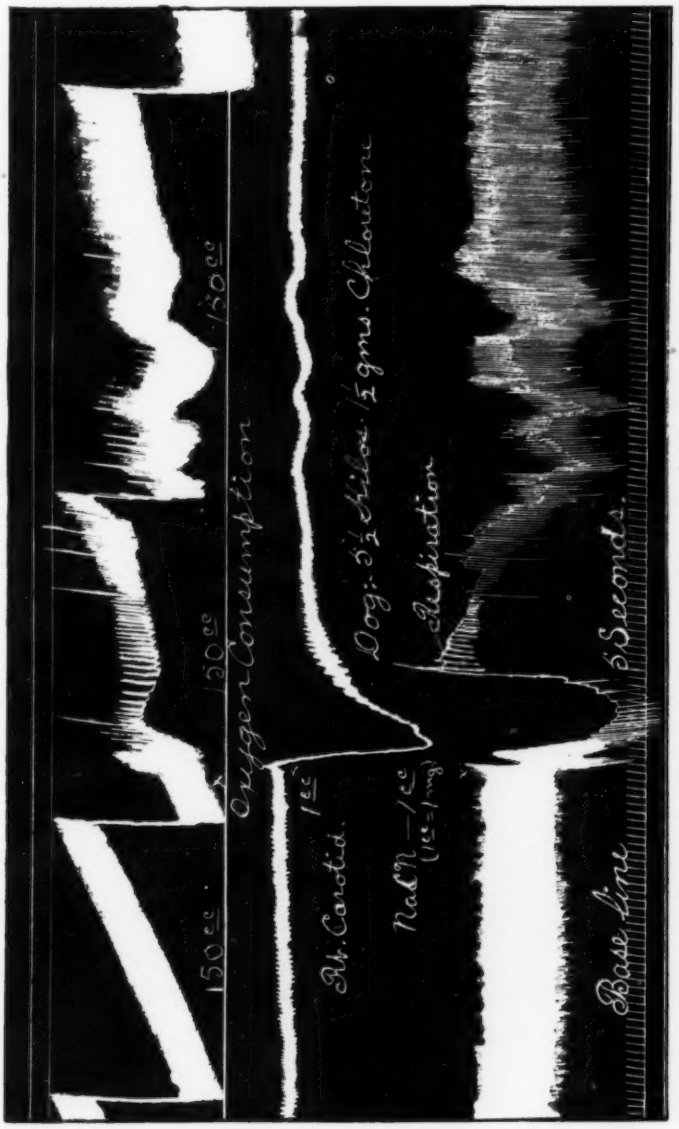

Fig. 8.

The gradual lowering of the vitality of the animal (perhaps from the prolonged anesthesia) in a long experiment may also show as a gradual progressive reduction in the amount of oxygen consumed per minute.

Fig. 5 illustrates a peculiar phenomenon brought out by drugs which cause marked constriction of the bronchioles. From this tracing it will be seen that

immediately after the intravenous injection of one milligram of arecoline hydrobromide there was a sudden drop in blood pressure (stimulation of the inhibitory endings of the vagi in the heart) and a marked decrease in the amplitude of the

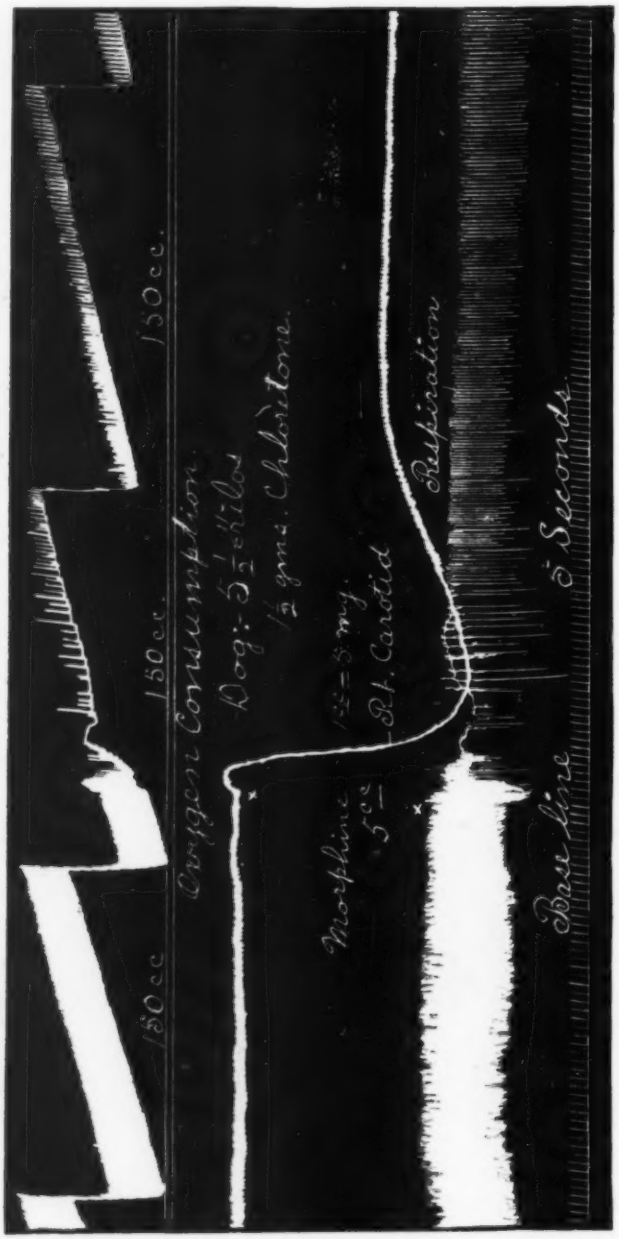

Fig. 9.

respiratory movements (tracing marked "respiration"). A brief increase in the intestinal contractions also occurred (vagus stimulation). The point which is mostly to be emphasized here, however, is the change in appearance of the oxy-

gen consumption record. The ascending curve of this tracing was suddenly decreased in amplitude (less air passing into and out of the lungs). This change might be due wholly or in part to a depression of the respiratory center, thereby causing a decrease in the respiratory efforts. It is not at all probable, however,

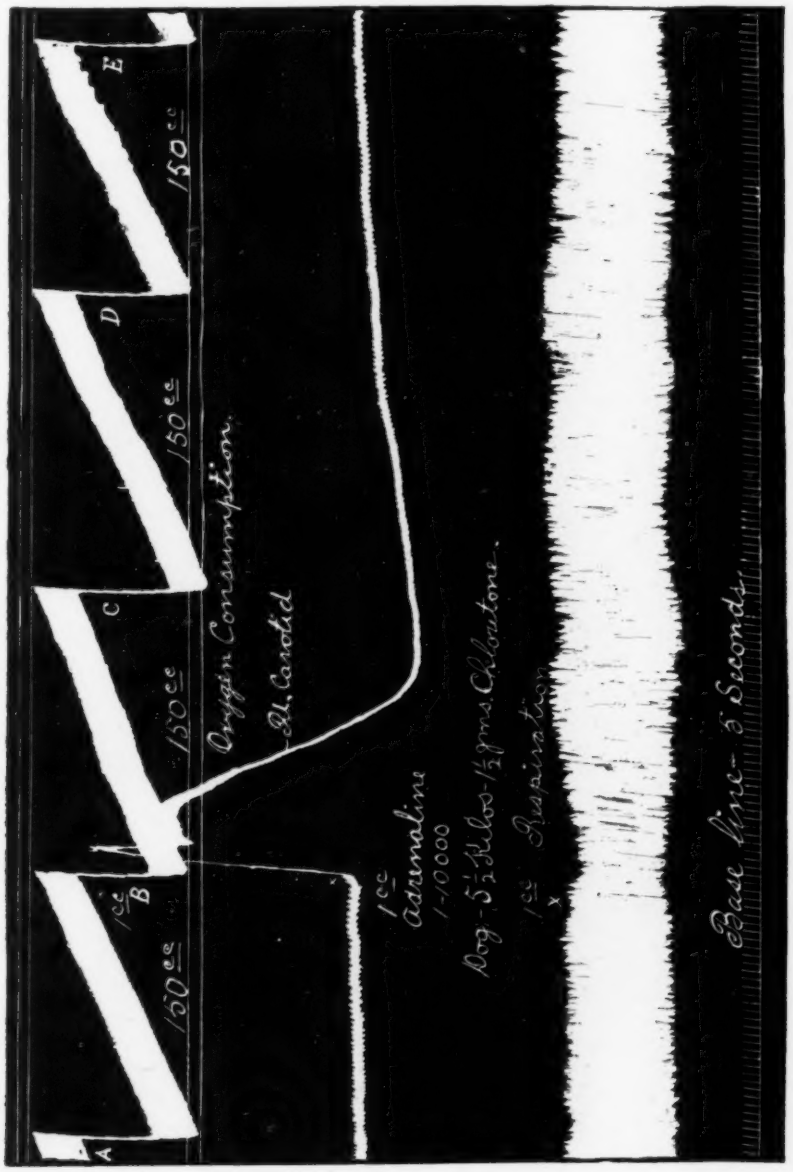

Fig. 10.

that this is the main cause for it can easily be shown experimentally that this dose of arecoline will greatly constrict the bronchioles and this lessens the volume of air which the lungs will hold. It seems evident therefore that this constriction of the bronchioles is mainly responsible not only for the decrease in

amplitude of the oxygen tracing, but also for the actual fall of the ascending limb of the oxygen record. This action of arecoline does not last long, however, and after a brief period the oxygen record again begins to ascend in the usual fashion. These results correspond exactly with the action of arecoline on the bronchioles. And this sudden change in the ascending limb of the oxygen record might be used as a method for studying the action of broncho-constricting drugs. It is possible that this intense broncho-constriction may hinder the absorption

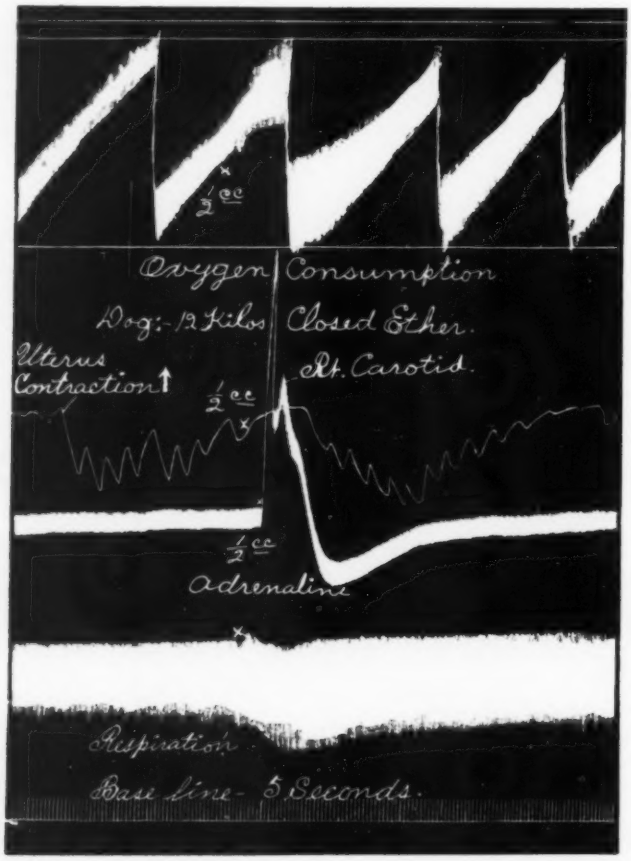

Fig. 11.

of oxygen or the excretion of carbon dioxide by the lungs, but the record does not perhaps show any such change in this case. And on the basis of other experiments (with different drugs), and since the animal was breathing a very high percentage of pure oxygen I am inclined to suspect that the broncho-constriction did not appreciably affect the gaseous exchange so far as the *mechanical actions* were concerned. This point is of interest in consideration of the dyspnea present during the paroxysmal periods of bronchial asthma. Fig. 6 shows the action of four milligrams of pilocarpine (in two doses) injected intravenously into the same animal. Here the broncho-constriction was distinctly present but less marked than after the arecoline. But following the

administration of the drug there is a very evident change in the rate of oxygen consumption which is increased. Possibly this is due to the great increase in the flow of the secretions as well as to the contractions of certain groups of smooth muscle which followed the injection of the drug. In this case although there is a moderate prolonged broncho-constriction produced by the drug there is evidently no lessening of the rate of oxygen consumption. This again is of interest in consideration of the dyspnea present during the paroxysmal periods of bronchial asthma.

In reference to these two records a further point should be mentioned. A number of observers, especially Haldane and Smith,[3] and Bohr[4] have suspected that gaseous exchanges at the surface of the lungs may be partly controlled by some secretory mechanism which may possibly be under nervous control. And Dreser has shown that in fishes pilocarpine will cause an increased secretion of oxygen into the swimming bladder. If either of these drugs possess any such action as is here indicated on either the absorption or excretion of oxygen or carbon dioxide by the lungs of dogs the records obtained by this method might be influenced by this action.

An interesting phenomenon is shown in Fig. 7 in which a dose of "ergamine" (β-iminazolylethylamine) was injected into a dog. A marked broncho-constriction must have been produced by the drug and this usually persists for a considerable length of time. The evidence of the constriction is seen on the oxygen record while the marked and prolonged fall in blood pressure shows that the drug was producing its usual effects. In spite of these changes, however, the rate of oxygen consumption undergoes but little change from the normal. This again reminds one of the changes in bronchial asthma. In this tracing the blood pressure fell to approximately one-third the normal level (mercury manometer) but this had only a minor influence on the oxygen record.

Figs. 8 and 9 show the action of sodium cyanide and morphine respectively on the rate of oxygen consumption. By comparison it will be seen that while in the cyanide tracing the blood pressure remained well up to the normal (after the primary fall), in the morphine record the pressure was only about one-half as high as it had been before the drug was injected. Furthermore the rate and depth of the respiration was more affected (in the latter part of the tracing) in the morphine experiment than in the cyanide record. Yet the changes in the relative rate of oxygen consumption were fairly comparable in the two cases. A comparison of Figs. 6, 7, 8 and 9 at once indicates that the rate of oxygen consumption in the animal organism is not only influenced in very different ways by various drugs but that within fairly wide limits the rate of absorption of oxygen by the lungs (and presumably the elimination of carbon dioxide) is largely independent of the height of the blood pressure, of the rate and depth of the respiratory movements and of the contraction or relaxation of the bronchioles. With reference to cyanides I may add that very small doses do not appreciably slow the rate of oxygen consumption, but on the other hand they appear, at least in some instances, to actually accelerate the consumption of the gas.[5]

In Figs. 10 and 11 I want to call attention briefly to a phenomenon which I have observed a great many times although in other instances I have failed to

obtain it. This consists in what appears to be a brief slowing down of the rate of oxygen consumption immediately after the intravenous injection of a fairly large dose of adrenaline. A large number of investigators have discussed the action of adrenaline on the metabolism of the body, especially in connection with the relation which the drug may have to the production of glycosuria, or to the oxidation of carbohydrates in the body. Among these may be mentioned Blum,[6] von Noorden,[7] Wilenko,[8] Edmunds,[9] Lusk,[10] Macleod,[11] and many others. I am inclined to believe, however, that none of these investigators have had in mind exactly the point which I mention here. It will be observed from the tracings that the first one (or two) saw-tooth notches following the injection (*intravenous*) of a good sized dose of adrenaline are longer than either the normal notches or the notches which follow later after the action of the drug wears off. While I have sometimes failed to obtain this result still I believe that under perfectly satisfactory conditions, and when the animal is strong and vigorous, this result should always be produced. I need not attempt any explanation of the phenomenon for the present. The vascular constriction over a considerable part of the body for a brief period might cause such a result. And the dilating action which the drug exercises on the bronchioles may also be concerned in some way for this dilation passes away as the action on the blood pressure ceases. It appears that the period of slower oxygen consumption (if the phenomenon is really due to this) corresponds more directly with the period during which the action of the drug is passing off, i.e., while the blood pressure is falling and for a few moments thereafter. Cannon[12] and his co-workers have recently emphasized the action which adrenaline has on muscular contraction and on the stimulation threshold of fatigued muscle. I should not be surprised if in the long run the phenomena observed by Cannon and his associates are found to be rather intimately related to the action of adrenaline on oxygen consumption which I have here indicated.

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STUDIES OF THE TISSUE REACTIONS TO VARIOUS PRODUCTS OF THE TUBERCLE BACILLUS.*

By Plinn F. Morse, M.D., and Ethel Stott.

THE histological structure of the tubercle is unique in that it is almost the only specifically characteristic tissue reaction to injury. Hosts of injurious agents cause lesions and tissue reactions more or less alike. The streptococcus, the colon bacillus, and various chemical substances all cause tissue reactions grouped under the head of various inflammatory phenomena but the specific presence of none of these agents can be affirmed or denied on the grounds of the tissue reaction alone. Even the spirocheta pallida, while its various lesions are often characteristic and capable of positive diagnosis from their histology, many times causes lesions not at all typical enough histologically to make them positively diagnosable. Lacking the organism itself, in the picture, as in actinomycosis or blastomycosis, or some product of its growth as the cyst of echinococcus, the histologist has practically two infectious conditions which he can positively diagnose from the appearance of the tissue reaction, namely, tuberculosis and leprosy.

The interest in this point of view was stimulated by our receiving in the laboratory some tissue from a patient who had had her face injected with paraffin for prosthetic purposes after mechanical injury. There appeared at the site of injection, lumpy tumors in the areolar subcutaneous tissue. These were removed and we were impressed by the fact that they were strikingly like young tubercles in structure. They consisted of avascular epithelioid granulations containing giant cells. Discussion of the subject with Dr. V. C. Vaughan, Jr., recalled the fact that he had held for a long time that the tubercle was the product of a foreign body reaction and not a reaction to a living organism primarily. This suggested the relationship of paraffin to the tubercle bacillus in that they were both waxy bodies; and this work was undertaken to study these reactions.

THE LITERATURE.

The mechanism of the formation of the tubercle has been a subject for investigation for a long time. Wyssokowicz,[1] cited by Masur,[2] found that tubercle bacilli were present and stainable in the tissues as long as one month after the injection and that epithelioid giant cells were grouped around them in the liver. Koch[3] found that dead tubercle bacilli caused aseptic pus upon subcutaneous inoculation. Buchner[4] believed that the albuminates of the bacteria were responsible for the lesions. Mafucci[5] did a large number of experiments inoculating killed tubercle bacilli subcutaneously with the result that the animals died several months later with a general marasmus, atrophy of all organs with stasis of lungs, liver, kidneys and spleen. There was a large quantity of pigment in the spleen. While Mafucci ascribed these deleterious results of the injection of dead tubercle bacilli to toxic

*From the Buhl Laboratory of Harper Hospital, Detroit.

substances, Prudden and Hodenpyl[3] worked with cultures which were carefully washed and then heated for a long time in water or five per cent glycerin. The dead bacilli were then freed from all water-soluble metabolic products. Subcutaneous injection caused aseptic pus, and intraperitoneal injection caused nodules on the serous surfaces similar to miliary tubercles but no caseation took place. When intravenously injected a part of the animals died of malnutrition in the third week and the rest died after longer periods. After three or four days, the bacilli were evenly distributed throughout the organs, lung, liver and spleen. From the fifth day on there formed in the lungs numerous white nodules which microscopically were typical of tubercles and contained bacilli which stained well. Similar pictures were found in the liver. Four to six weeks after the inoculation, the lungs of the animals were exactly similar to those in which virulent bacilli had been introduced. The bacilli began to disappear in the later weeks and became granular. They obtained like results by spraying the trachea with dead cultures.

From these findings, Prudden and Hodenpyl concluded that apparently the protoplasm of tubercle bacilli contained poisons which cannot be extracted by the usual methods but which are set free in the animal body and act with positive chemotaxis on the animal tissue causing the formation of the nodules.

Straus and Gamaleia[7] record substantially the same results as Prudden and Hodenpyl, finding that dead tubercle bacilli could form perfect tubercles.

Only once in the literature am I able to find the idea specifically stated that the tissue reaction to the tubercle bacillus is that of a foreign body. Vissman,[8] while agreeing in the main with Prudden and Hodenpyl, and with Straus and Gamaleia in regard to the lesions produced, showed that these lesions were the same whether the bacteria were killed in their own water of condensation or by heating in Ehrlich's fuchsin solution and comes to the conclusion *that the tubercle bacillus is a powerfully stimulating foreign body.* Like Prudden and Hodenpyl, Vissman was not able to produce caseation.

Masur[2] reviews the previous literature and sums up the whole matter in the following conclusions:

1. Dead tubercle bacilli injected intravenously cause either a severe general disease or death of the animal.

2. There are constantly found in injected animals, tubercle-like nodules in the lungs with giant-cells but without caseation, further, a more or less severe chronic interstitial pneumonia.

3. A part of the animals used show catarrhal pneumonia processes in the lungs, inflammatory changes of varied sort in the kidneys, pigment deposits on the spleen and disease of the vessels.

4. It is not important in what manner the bacilli are killed.

5. The organ changes as a whole, are apparently dependent upon the influence of toxic substances which are liberated by the disintegration of the tubercle bacillus.

Abel[9] believes that a body was set free from the bacteria by the tissues which acted as an irritant and caused the tubercle.

Gamaleia[7] occasionally obtained some caseation and believed that caseation did not occur because the animals died too quickly. He believed that the

bacilli, in addition to their chemotactic effect on the body cells, contained a destructive poison.

Grancher[10] showed that the effect of injected killed avian tubercle bacilli in rabbits was to cause typical peritoneal tuberculosis.

Koslenitsch[12] showed that injection of sterilized tubercle bacilli into the anterior chamber of a rabbit's eye either caused pus or after four to eight weeks, nodules in the iris which consisted of epithelioid tissue and a few giant cells. The nodules did not caseate and contained bacilli.

Weyl[13] was apparently the first to attempt an analysis of the effects of chemically separate constituents of the dead bacilli. He obtained two bodies from the tubercle bacillus, one of indifferent nature which had the tinctorial properties of the bacteria and the other a toxomucin which upon subcutaneous injection caused dry necrosis of the skin.

Grancher and Martin[14] found in their efforts to immunize animals, that after injection of very old cultures of tubercle bacilli the animals frequently died of parenchymatous nephritis. This result, as well as the occasional appearance of paraplegia and cachexia was ascribed to a toxic substance in the body of the bacillus.

It is evident from these references that the preponderant opinion of investigators has been that the histological lesion caused by the tubercle bacillus was due to a poison liberated from the body of the bacillus by action of the tissue cells. To test these ideas, the bacilli were grown in mass culture on ten per cent glycerin bouillon in three or four liter Erlenmeyer flasks. After a thick pellicle had formed the flasks were autoclaved and the growth obtained by filtering off the bouillon and washing the residue with cold water. The sterile growth was dried over sulphuric acid in the desiccator and then ground to a powder. The powder was extracted in two fractions. Ether was first added and the mixture kept in the ice box for twenty-four hours and then the ether filtered off. Evaporation of the ether extract yielded a thick brown oil which soon became green by exposure to light and air. The residue after ether extraction was dried in air and extracted in a reflux condenser in boiling alcohol for one-half hour, filtered hot and the filtrate evaporated. The residue consisted mostly of protein germ substance and remains to be investigated.

The evaporated filtrate from the boiling alcohol extract yielded a light yellow wax-like substance darkened slightly to a reddish-brown on standing (impurities?). These two lipoid substances were tested separately for their effects upon rabbits, and white rats. Rats were injected with quantities varying from 0.25 to 1.0 c.c. intraperitoneally and in the groin. The animals were killed at intervals of three days to four weeks after injection by putting them in a small chamber and turning in illuminating gas. They stopped breathing within thirty seconds, although the heart was usually beating at the autopsy.

The dark, viscous oil extracted by cold ether produced no lesions at any time. It was injected intraperitoneally and subcutaneously in the rat's groin and after the initial inflammatory reaction had subsided the tissue was normal again. Intravenously in rabbits, doses short of the production of severe pulmonary embolism produced no lesions whatever.

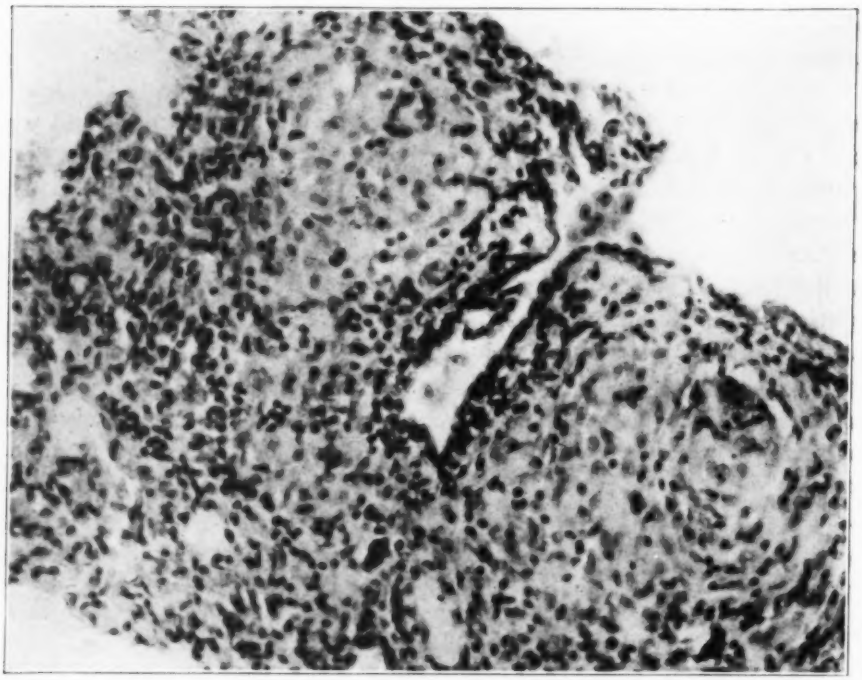

Fig. 1.—Rabbit's lung four weeks after intravenous injection of wax from tubercle bacillus. Epithelioid tubercles with giant cells.

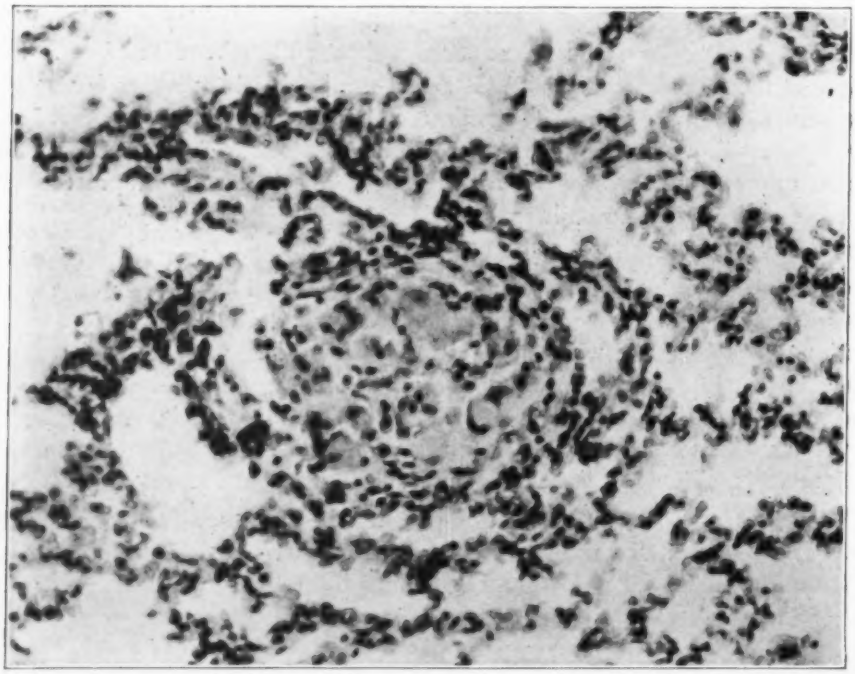

Fig. 2.—Epithelioid tubercle four weeks after intravenous injection of waxy substance.

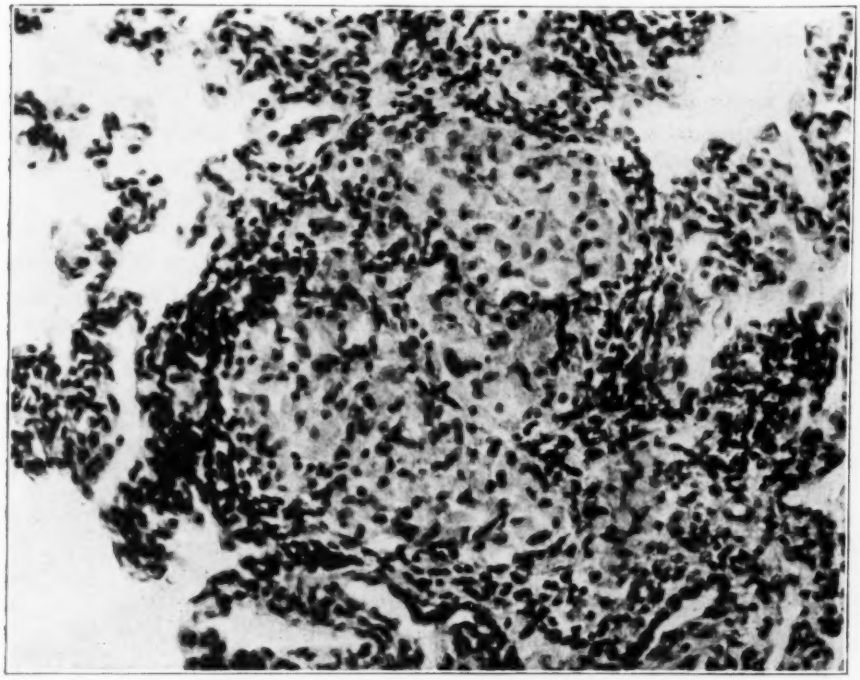

Fig. 3.—Epithelioid tubercle with giant cells in rabbit's lung four weeks after intravenous injection with waxy substance from tubercle bacillus.

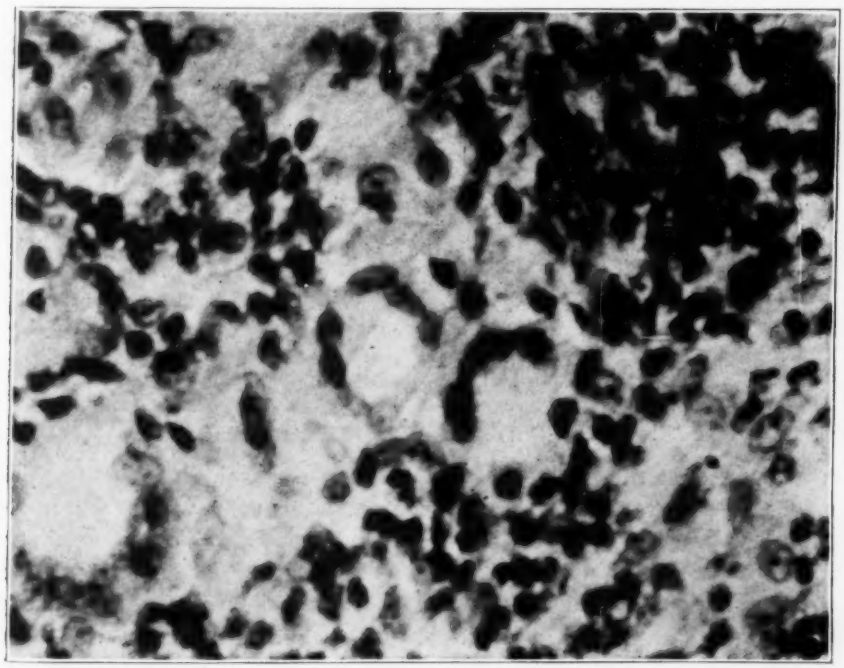

Fig. 4.—Giant cell formation in epithelioid lesion due to waxy substance from tubercle bacillus.

Effects produced by the waxy substances can be divided into early and late lesions. The wax was injected in doses of 0.25 to 1 c.c. in the same manner as the oily substance. The initial vascular reaction was not great, but in about twenty-four hours the site of injection was found to be indurated and swollen and if opened at this time, a serous exudate, containing many eosinophile cells, had formed around the wax droplet.

The substance was introduced into the lung of the rabbit both dissolved in olive oil and warmed until fluid enough to flow through a syringe. In all cases, the wax was sterilized carefully and injected aseptically. Most of the

Fig. 5.—Nodules in right groin two weeks after subcutaneous injection of tubercle waxes.

substance lodged in the capillaries near the outer margins of the lung in the lower lobe as in experimental fat embolism. At times, where a large dose was given too rapidly, a large enough embolus was introduced to produce infarction of a portion of a lower lobe; usually, however, a few hours after injection examination showed only intense edema and blanching of the margins. In twenty-four hours polymorphonuclears were abundant and the tissue blased with eosinophiles when stained with hematoxylin and eosin. The wax appeared not to produce any marked degree of thrombosis. Once in a lesion, three days' old with a good sized infarct, an induced thrombus was observed.

The lesions in the lung were most interesting at the end of three days. By this time, endothelial proliferation was very active and whole areas of the lung tissue were filled in solid with endothelium, polymorphonuclears, and eosinophile leucocytes. There was considerable fibroblastic reaction also (Fig. 8, A). The endothelium on the capillaries which were distended by the wax first swelled up and projected into the lumen soon obliterating the capillary and forming a syncytial mass with many dividing endothelial cells (Fig. 8, A and B). This method of occlusion of capillaries is interesting in view of the fact that it gives a possible explanation of the avascularity of a tubercle.

Fig. 6.—Intraperitoneal lesions two weeks after injection of tubercle waxes.

The usual method of vascular obliteration assumed heretofore has been that the growing bacillus quickly injures the endothelium of the capillaries in the infected area, leading to thrombosis with subsequent organization. This view does not seem justified inasmuch as the peculiar type of granulation tissue characteristic of tuberculosis is avascular from the start and consists primarily of endothelial whirls. I believe that whatever tendency to the formation of vascular capillary buds there may be is subverted by the unusual stimulus driving the endothelium to proliferate, thereby obliterating the endothelial cords and changing them to a syncytial mass (Fig. 8, A).

The development of the whirls of the endothelial cells now progresses until the wax is entirely walled off and the lesion reaches its full development in about four weeks (Figs. 1, 2, 3, and 4).

There were found in the subcutaneous lesions, lumpy masses filled with yellow purulent material surrounded by avascular epithelioid granulation tissue containing giant cells. The contents of these softened areas were never caseous but either purulent or filled in solid in the smallest ones with granulation tissue of the characteristic type (Fig. 5).

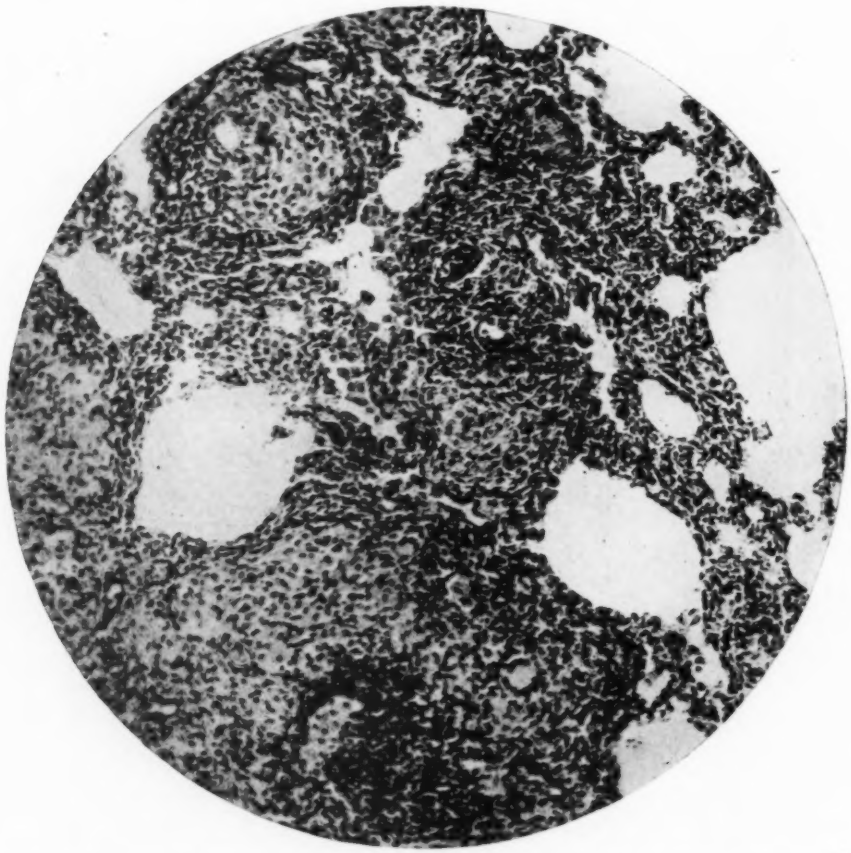

Fig. 7.—Low power. Rabbit's lung four weeks after intravenous injection of wax-like substance of tubercle bacillus.

In the peritoneum (Fig. 6) the lesion presented a typical appearance at the end of two weeks. The omentum was stretched transversely in a roll below the stomach or twisted and adherent to various portions of the abdomen. There were numbers of little white spots like miliary tubercles which microscopically were found to consist of epithelioid whirls without blood vessels. The surface of the liver showed tiny white points and the lower margin was covered by an organized exudate.

The resulting picture in the rabbit's lung, after four weeks, was most constant (Figs. 1, 2, 3, 4, and 7). All the signs of acute inflammation were gone.

Fig. 8.

A. Endothelial syncytium. Showing dividing endothelial cell and eosinophiles. Early lesion.
B. Capillary after injection of wax-like substance showing beginning endothelial obliteration of vessel.
C. Late lesion. Avascular epithelioid tubercle four weeks after injection of wax-like substance.

(Morse: Tissue Reactions to Various Products of Tubercle Bacillus.)

The leucocytes and eosinophiles had almost disappeared. Scattered irregularly throughout the lung were typical structures consisting of avascular epithelioid whirls of granulation tissue (Figs. 2, 3, and 7). There was an increase of connective tissue in the lung as a whole, evidenced by the thickening of the septa. The areas did not undergo caseation and were well walled off by young fibroblasts (Fig. 8, C), as one would expect in a non-progressive condition. At this time, giant cells were numerous (Figs. 3 and 4) formed by fusion of the epithelioid cells. The wax had not yet disappeared, but was still present in giant cells and in tissue spaces surrounded by avascular granulation.

The animals never showed cachetic phenomena or other abnormalities through the experiments. The wax is apparently totally devoid of toxic properties. Whether either the oily substance or the wax is a single substance or a mixture of several substances has not been determined. The word wax has been used entirely in the physical and not in the chemical sense throughout this article.

These results have suggested the problem of more careful chemical separation of all the constituents possible from the tubercle bacillus with separate testing of their tissue reactions.

The literature on the chemical side of the subject is not given here since it does not pertain to this mechanical portion of the "Arbeit." In these lesions, like all those produced by the whole dead bacillus caseation never occurred. Whether actual necrosis can be accomplished in this way or not is doubtful. The ability to cause the tissue to die is possibly an attribute of the living bacillus. The role of the possibly unsaturated fatty substances of the bacillus in preventing the digestion of the dead tissue by leucoproteases as suggested by Petersen and Jobling is a promising subject for investigation.

The frequency of appearance of tubercles in organs having a large amount of endothelium in their structure, such as lymph nodes, lung, liver and spleen, may be something more than a mechanical coincidence. Klotz[11] has shown that olive oil used as a menstrum for cholesterol injections has a tendency to cause selectively a proliferation of the endothelium.

It seems clear, however, from this short series of experiments, that the tubercle bacillus produces a microscopically characteristic lesion not because it is a living particulate protein, but because its waxy substances act as a peculiar type of foreign body.

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TONSILLECTOMY DURING THE COURSE OF ACUTE RHEUMATIC FEVER.*

By Roger S. Morris, M.D., Cincinnati.

THERE is a considerable group of workers who hold that the tonsils should be removed as a routine procedure in all cases of chronic infectious arthritis, in which the origin of the infection is at all obscure. The law of probabilities is here relied on, for it is the experience of many that the tonsils harbor the focus of infection more often than other parts of the body. And it is probably safe to conclude that all who painstakingly follow this rule in chronic infectious arthritides have seen brilliant results, not in all cases nor even in a majority of them, but nevertheless in a sufficient proportion to justify the procedure. Even when the operation fails to bring the hoped for relief, we have no proof, in the absence of animal inoculations, that it was not indicated, for the work of Rosenow and others has shown the frequency with which multiple foci of infection are encountered. So, too, many of us are having tonsils removed in cases of recurring endocarditis, myalgia, and so on.

The present paper deals, however, not with tonsillectomy in chronic infectious arthritis, but with its value in acute rheumatic fever, a field in which, so far as I am aware, the indications for the procedure, its dangers, and the possible benefits to be derived from it are not so generally appreciated. Our own experience is limited to a small but encouraging group of cases.

It is a generally accepted clinical fact, first noted by Eyerlen[1] in 1798, that acute rheumatic fever is very frequently preceded by acute tonsillitis. The association is of such common occurrence that for years the tonsils have been looked upon as one of the portals of entry of the infection. Not only in primary attacks of acute rheumatic fever, but also in subsequent attacks, it is a common experience to obtain a history of sore throat or acute tonsillitis as the initial event in the illness. The arthritis or endocarditis, chorea or erythema nodosum then follows in a comparatively short time after the onset of the throat symptoms. In other words, it is often evident that the rheumatic cycle begins with manifestations of tonsillar infection.

The frequency with which acute rheumatic fever is preceded by sore throat is variously estimated. Pribram[2] finds it in only 1.5 per cent of cases. Dieulafoy[3] says it occurs in at least a third of all cases. Others find evidence of tonsillitis, acute or chronic, in as much as 80 per cent of the cases.

It is evident from a perusal of the literature and also from clinical experience that the history given by the patient is often unreliable. The local symptoms in the throat are so mild oftentimes that little attention is given to them. Furthermore, there may be serious disease in or about the tonsils, with little or no local manifestations. Simple inspection of the throat may not be sufficient in many cases to detect the trouble. It is significant that recent writers, employing more thorough methods of examination of the tonsils, find these organs diseased

*From the Cincinnati General Hospital.

as a rule in a much higher percentage of cases of acute rheumatic fever than was formerly the case.

It is not presumed, of course, that the tonsils are the only portal of entry or depot of infection in acute rheumatic fever. Indeed, there is good clinical evidence to the contrary. Abrasions in the mucosa of the nose have been followed by acute polyarthritis with or without endocarditis in a number of instances. Such cases are recorded, among others, by M. Senator,[4] de Stella, and von Kronenberg. Goadby[5] and others report cases of acute polyarthritis originating from pus pockets about the teeth. Mackenzie[6] believes the virus may gain access to the body of children at times by way of the bronchial or intestinal mucosa. But all are apparently agreed, at the present time, that the most frequent portal of entry and focus of infection is to be found in the faucial tonsils.

Given, now, a case of acute articular rheumatism, there can be no hesitation regarding the proper medicinal therapy, i. e., salicylic acid. Fortunately or unfortunately, the joint symptoms of the great majority of cases respond promptly to this drug or its derivatives. But the question naturally arises, is one's duty to the patient discharged when the acute arthritis has been controlled? Is the patient, in other words, fully protected by the relief of his symptoms—his joint pains? It is universally known, of course, that such is not the case. In no disease are complications of commoner occurrence than in acute rheumatic fever.

Osler's[7] statements regarding the frequency of complications in rheumatic fever give a good general idea of their prevalence, though there is probably considerable deviation from the general averages at times, endocardial infection, for example, being apparently much commoner some years than others. Endocarditis, resulting eventually in chronic valvular disease, occurred in more than 50 per cent of 889 cases analyzed by Church,[8] while it was found in about the same proportion of 116 of Mackenzie's cases in the first attack, in 71 per cent during the third attack. Romberg found 58.5 per cent of 670 cases of endocarditis attributable to rheumatism.

Pericarditis, while not unusual, is met with much less often than endocarditis.

Myocardial changes are almost inevitable with either peri- or endocarditis.

Pneumonia and pleurisy occur in about 10 per cent of cases.

Other complications are rarer. But it is clear that while salicylic acid is usually efficacious in relieving the arthritis, it is practically powerless in controlling the infection in other parts of the body.

It is such considerations as these which have led many to look upon the complications not as such but rather as parts of the disease picture. To Gürich,[9b] I believe, belongs the credit of emphasizing the probability that in acute rheumatic fever joint symptoms and the so-called complications are all the result of metastatic infections from a primary focus elsewhere in the body. And if one is to accept this conception of the disease, the therapy can no longer consist merely in the administration of salicylates, the application of local treatment to the joints, and rest in bed.

The first to attempt systematic treatment of acute rheumatic fever through the tonsils appears to be Gürich,[9a] who, in 1904, reported seventeen cases. Twelve of his patients had more or less severe tonsillitis, and four had peritonsillar abscesses preceding the appearance of the acute arthritis. In fourteen of the

cases plugs were seen in the crypts. He cites a few illustrative cases of acute rheumatic fever which were cured rather promptly by local treatment of the tonsils, but he does not say what proportion of the patients were benefited. Enucleation of the tonsils was not done.

In the following year, Gürich[9c] published a small monograph dealing with one hundred and forty cases of arthritic affections, subjected to the so-called tonsillar therapy. Fifteen of these cases are discarded, as the patients began treatment but left for one reason or another before the treatment was completed. Of the remaining one hundred and twenty-five cases, twenty-three were entirely unaffected by treatment of the tonsils, which consisted, as in his previous report, of making parallel incisions through the tonsils as early as possible, and within one to two weeks, curetting away what remained of the tonsils. Ninety-eight of the patients, he says, were finally cured by this treatment. His series, apparently, was not limited to cases of acute rheumatic fever but included also cases of chronic infectious arthritis. The proportions of these diseases are not stated.

In 1908, Rosenheim[10] reported ten cases of acute articular rheumatism in whom tonsillectomy was performed, two to six or more weeks following the onset of the acute arthritis. All were improved, the arthritis subsiding promptly in a number of instances. From the abstracts of histories, it appears that in no case did endocarditis develop subsequent to operation. Streptococci were found in all of the tonsils removed, in the majority of instances in pure cultures. No early operations were performed. As far as I can discover, Rosenheim is the first to enucleate the tonsils in acute rheumatic fever.

Hess,[11] in the following year, called attention to the frequency of tonsillitis in acute rheumatic fever. Acute follicular, phlegmonous or catarrhal tonsillitis or quinsy may usher in the attack. The author also emphasizes the importance of chronic inflammation of the tonsils in the etiology of the disease. Hess does not resort to surgical treatment of the tonsils in all cases. He gives no statistics of his own experience.

Schichhold,[12] in 1910, reported a series of seventy cases of acute rheumatic fever treated by Gürich's method. In practically all of his cases, thorough examination of the tonsils showed evidence of disease. He called attention to the importance, too, of looking for other foci of infection, such as the sinuses or teeth. Schichhold made incisions in the tonsils, as a rule, and later removed them, using Gürich's method. Subsequent to the incision, there was often immediate improvement in the joints, with a return of the symptoms in two or three days,—a reaction which Gürich had noted. At the time the joint symptoms subsided, the temperature usually fell; there was then a rise for two or three days, followed again by a fall. The second operation caused little reaction. The author strongly recommends the tonsillar treatment of acute articular rheumatism. His impression is that recent endo- and myocarditides heal more quickly after the operation on the tonsils.

Curschmann[13] cites cases of septicemia and of acute nephritis secondary to tonsillar infections, but he believes Gürich and Schichhold go too far in saying

that practically all cases of acute recurring polyarthritis should undergo tonsillar treatment.

A few other case reports are to be found in the literature, which support the frequency of the tonsillar origin of acute rheumatic fever.

The tendency has been, I believe, to defer the operation until the patient has recovered from his illness—to perform what might be termed an interval operation, both as regards arthritis and tonsillitis. *A priori*, it would seem that there are certain dangers in deferring the operation. For, if the tonsils are the focus of infection, their presence means that there is always a possibility, in fact, a probability, of metastatic infections of the heart or, less often, of other organs. It would, therefore, appear that, in cases of acute rheumatic fever where there is a definite history of tonsillitis during the present or a previous attack of arthritis, or where the tonsils are found to be diseased, they should be removed as soon as the operation can safely be performed.

To determine whether—and when—to operate, a number of factors must be considered. The first and most important desideratum is the service of a skilled throat specialist, who shall not only perform the operation but also determine whether the acute process in the tonsils (if it be of recent date) has subsided sufficiently to make operation reasonably safe. Judgment is often difficult, as the occasional occurrence of endocarditis following tonsillectomy demonstrates. (Cases VII and VIII.*) But from a limited experience, we feel that, with the subsidence of outward evidence of acute inflammation of the tonsils, the danger of allowing them to remain in the body probably exceeds the danger of their removal. In all cases, we believe, at the present time, that an attempt should be made to relieve the joint tenderness by full doses of salicylates, before the operation is attempted. A certain amount of freedom of movement is necessary after the operation, to enable the patient to expectorate, etc.; during the height of an acute arthritis, pain on moving would often prevent the necessary changes in position. The condition of the patient also determines the choice of anesthetic, and various factors again enable one to choose between general and local anesthesia.

The following brief abstracts of histories are cases in point:

CASE I.

F. B., hospital No. A-2009, a white male, single, aged 19, a furniture worker, was admitted to the North Medical Service of the Cincinnati General Hospital, March 20th, 1916, complaining of "rheumatism in the legs."

The family history was unimportant.

Past History.—The patient has had sore throat almost every winter for several years.

Present Illness.—Two days ago, the patient first began to suffer with pain in the legs and hips. He was unable to walk and was brought to the hospital in an ambulance.

Physical Examination.—The patient is a strongly built man. His tonsils are hypertrophied and reddened and the cervical glands are enlarged but not tender. The knees are swollen and show mottled erythema. There is exquisite tenderness in the knees. There is slight swelling of the ankles about the outer malleoli. The heart is normal.

The urine shows a faint trace of albumin and a few granular casts.

The Wassermann reaction is negative. Blood culture is sterile.

The temperature rose to 103 the day after admission.

*It is quite possible that in each of these patients endocardial infection had occurred prior to operation, though definite physical signs of valvular trouble were not present until after operation.

The symptoms were relieved by salicylates and the temperature became normal on March 23rd. Tonsillectomy was performed on March 29th. There were no other joint symptoms. The patient was discharged well, on April 6th.

CASE II.

G. W., hospital No. 5876, a white male, single, aged 30, a keeper of a market stand, was admitted to the North Medical Service of the Cincinnati General Hospital on November 11th, 1915, complaining of "rheumatism."

Family history is unimportant.

Past History.—The patient has had sore throat in the past and the tonsils were "removed" about twelve years ago. The patient had his first attack of acute articular rheumatism eleven years ago. He was in bed seventeen weeks at this time and says that every joint in the body was affected except the spine. The arthritis began in the feet and worked upward until the jaws were involved. He has had no cardiac symptoms. He denies venereal infection.

Present Illness.—The onset was acute on November 6th, 1915, with pain and swelling of the ankles. Within the last four days the knees and shoulders have become involved.

Physical Examination.—The patient is a strongly built and well nourished man. Pyor-

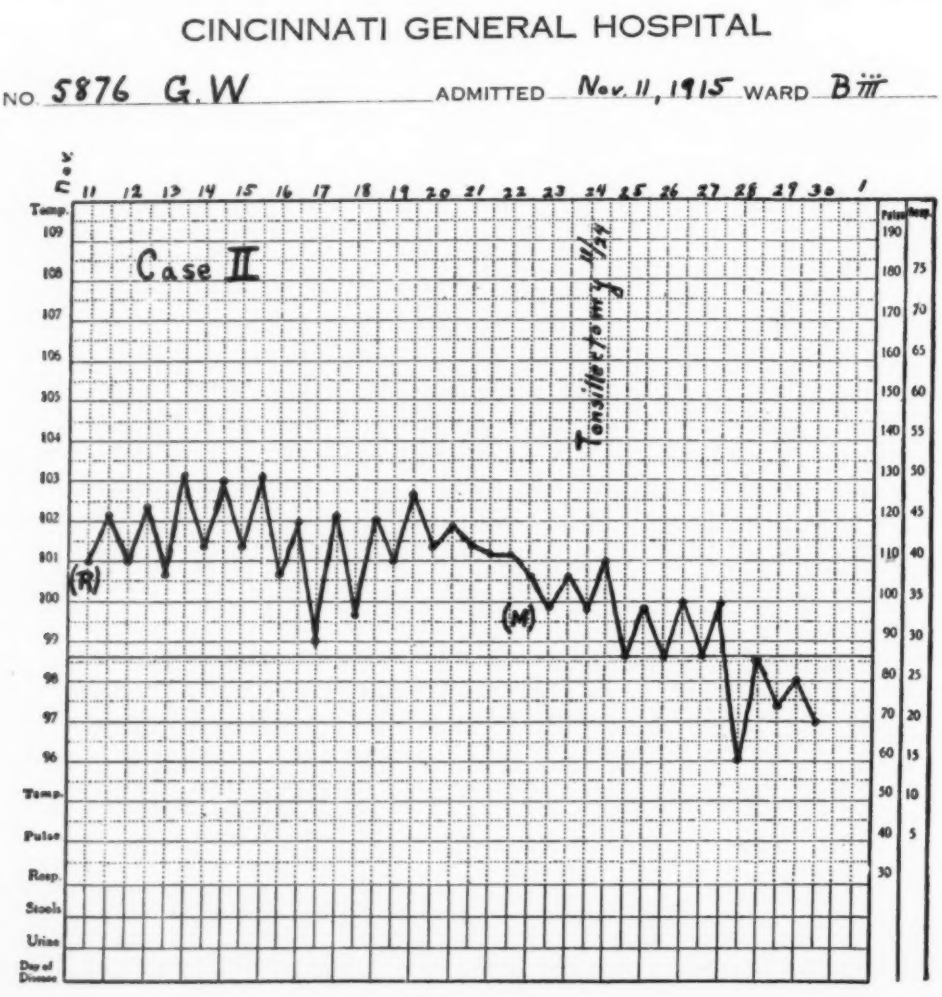

CHART I.

rhea alveolaris is present. There is a general reddening of the mucous membrane of the throat. Over the left tonsil which is large and ragged, there is a thin, grayish exudate. The right tonsil is also red and enlarged with a protuberance having a crater in the center. The pharynx is reddened and a small amount of purulent secretion is present. The cervical glands are not swollen.

Heart.—The point of maximal impulse is 9.5 cm. to the left of the median line in the 4th interspace. Relative dullness extends 3 cm. to the right in the 4th, and 12 to the left in the 4th. A blowing systolic murmur, which almost replaces the first sound, is audible at the apex and is transmitted to the axilla. The pulmonic second sound is accentuated. The blood pressure is 120 systolic, 80 diastolic.

There is swelling of each knee with increase in local temperature. The left knee is extremely tender to the touch. There is tenderness in the left hip.

The urine is negative except for the presence of a few finely granular casts. The throat culture is negative for diphtheria bacilli. The leucocytes number 16,500 of which 78% are polynuclear neutrophiles. A blood culture on November 14th showed streptococcus viridans in pure culture.

Local treatment improved the condition of the throat very much by November 14th. The joint symptoms responded quickly to salicylates. The temperature nevertheless remained elevated, being between 101 and 102.5 and the leucocytosis persisted.

Tonsillectomy was performed November 24th. The temperature reached normal for the first time on the following day, and after the third day it remained normal (see Chart I).

The patient was discharged December 2nd, 1915, feeling well. In July, 1916, the patient was seen at his stall in the market and says he has remained in good health.

CASE III.

H. H., hospital No. 6619, a colored male, single, aged 48, a laborer, was admitted to the North Medical Service of the Cincinnati General Hospital, December 14th, 1915, complaining of pain in both legs and arms and of headache.

The family history is unimportant.

Past History.—For twelve years the patient has had frequent attacks of sore throat. He had acute arthritis 18 years ago and at this time was in bed for two months. Thirteen years ago the patient had a chancre. He admitted having had two or three attacks of gonorrhea; the last one was fifteen years ago. He drinks alcohol to excess.

Present Illness.—(Unfortunately the notes of the present illness are lost.)

Physical Examination.—The patient is a well nourished man. The teeth are in poor condition. There is marked pyorrhea. The soft palate and uvula are moderately injected. The anterior pillars are much reddened. The tonsils are enlarged and hyperemic. The pharynx is injected. The patient complains of difficulty in swallowing. The upper cervical glands are palpable.

The heart is normal.

There is pain in both knees and elbows on motion.

The urine is negative. The Wassermann is strongly positive. On December 21st the leucocytes numbered 8,800, of which 77% were neutrophiles.

The temperature has been between 100 and 102.

Tonsillectomy was performed December 22nd, and deeply imbedded tonsils were removed. Within two days after the operation the temperature became lower and did not rise above 99.6. January 7th, the joint symptoms had disappeared and the patient was doing light work in the ward.

A blood culture made at the height of the disease was sterile. Treatment with mercury and iodide was begun December 16th. The patient was discharged February 16th much improved.

CASE IV.

F. E., hospital No. 6889, a white male, single, aged 27, a baker, was admitted to the North Medical Service of the Cincinnati General Hospital, December 26th, 1916, complaining of "rheumatism."

The family history is unimportant.

Past History.—The patient has had seven attacks of acute rheumatic fever. The first attack was fifteen years ago and the patient was in bed six months; the second attack

was in 1901, when he was in bed nine months; the third attack in 1902, in bed four months; the fourth attack in 1905, in bed five months; the fifth attack in 1907, in bed three and one-half months; the sixth attack in 1910, in bed two months. The total time in bed in the six previous attacks was twenty-nine months. The patient says he has been subject to mild attacks of tonsillitis, but he thinks they have had no relation to the rheumatic attacks.

Present Illness.—The present illness began December 17th, 1915, with pain in the great toe of the left foot. The following day he complained of pain and swelling in both knees and was unable to walk. The next day he suffered with pain in the back and now complains of pain in the right shoulder, wrist and knuckles and in the right knee.

Physical Examination.—The patient is a well nourished man of moderate size. Pyorrhea alveolaris is present. The soft palate, uvula and tonsils are moderately injected. The cervical glands are palpable.

Heart.—The cardiac dullness extends 11.5 cm. to the left in the 5th space, 4 to the right in the 4th space. A systolic murmur replaces the first sound at the apex and is transmitted to the axilla. The pulmonic second is accentuated.

The affected joints are red, swollen and very painful on passive motion.

The temperature is between 101 and 103. There are 18,800 leucocytes, with 85% of polynuclear neutrophiles. Two blood cultures on December 26th and January 2nd were sterile. Gonococcus fixation test is negative. Wassermann test is negative. Urine is normal.

CINCINNATI GENERAL HOSPITAL

NO. 6889 F.E. ADMITTED Dec. 26, 1915 WARD BT

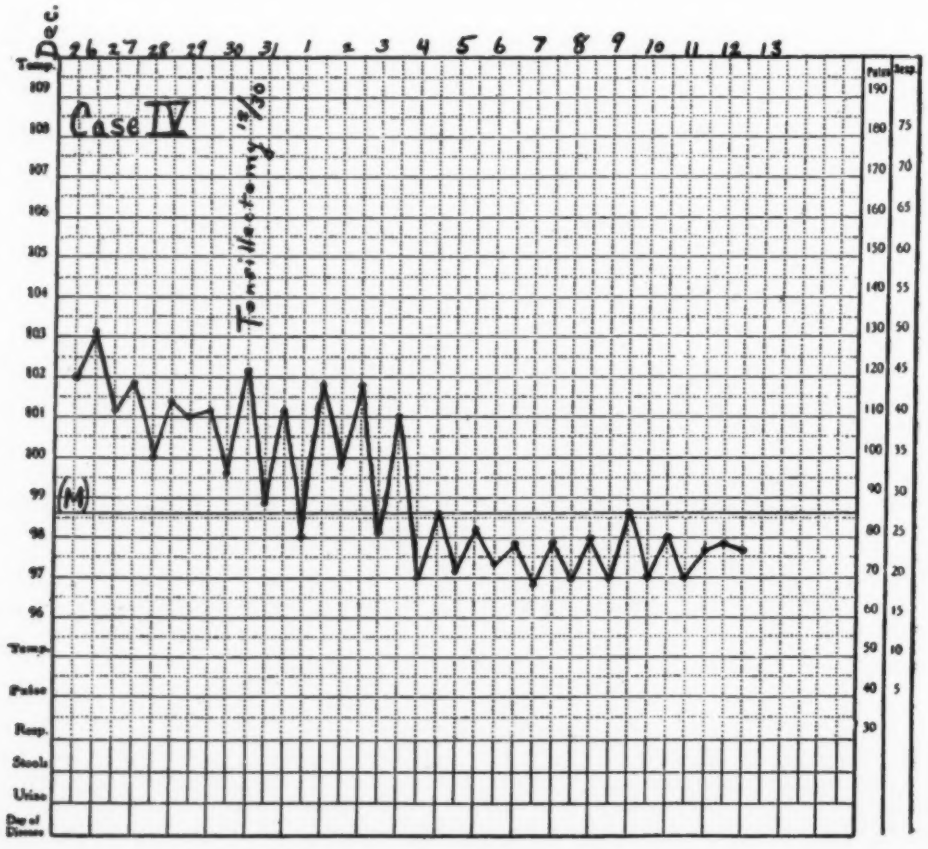

CHART II.

Tonsillectomy was performed December 30th. The temperature reached normal the fifth day after operation and remained so (see Chart II). The pain in the joints was much less marked by January 5th and on January 25th the patient was discharged, markedly improved.

X-ray plates of the teeth showed no abnormalities.

CASE V.

S. N., hospital No. A-5976, a white woman, single, aged 20, a shoe worker, was admitted to the North Medical Service of the Cincinnati General Hospital, September 5th, 1916, complaining of pain in the knee and finger.

The family history is unimportant.

Past History.—The patient has had frequent attacks of tonsillitis, and in the last four winters she has had an annual attack of quinsy and has been ill about two weeks with it as a rule. She has never had rheumatism before the present illness. She complains of slight dyspnea on exertion and occasional palpitation.

Present Illness.—On the night of August 31st, the patient complained of headache and soreness in the knee. She went to work the following day, but on September 2nd her physician sent her to bed. There have been no sweats. There have been soreness and stiffness in the knee and pain in the third finger of the left hand. There has been some nausea and vomiting.

Physical Examination.—The patient is a well developed, well nourished, rather anemic young woman. She is a mouth breather. The lips are thickened, the upper incisors prominent. The tonsils are hypertrophied, and the tonsils, pillars and uvula are reddened. There are no palpable glands in the neck.

The heart is somewhat enlarged, measuring 3.5 cm. to the right and 10.5 to the left. The point of maximal impulse is 8.7 to the left in the 5th space. The pulmonic second is accentuated, but no murmur has been heard over the heart. Blood pressure is 120 systolic, 60 diastolic.

The right knee and ankle are slightly swollen and the skin is warmer than on the left side; there is exquisite tenderness. There is also tenderness of the left third finger.

The urine is normal. The leucocytes are 20,000. Wassermann test is negative, as is also the gonococcus fixation test.

The Dental Service reported the teeth in good condition.

Tonsillectomy was performed September 9th. The patient had previously been given salicylates and alkaline drinks. Mouth temperature has not been above 99.6, pulse 100, respirations 26. Four days following tonsillectomy the temperature, pulse and respirations were normal.

The patient was sent to a convalescent home, September 16th, much improved.

CASE VI.

H. S., hospital No. A-788, a white male, single, aged 34, a machinist, was admitted to the North Medical Service of the Cincinnati General Hospital, February 2nd, 1916, complaining of "rheumatism from the hips down and inability to walk."

The family history is without interest.

Past History.—The patient says he has had sore throat every year since he was eighteen years old. The last three or four years he has had quinsy each year, with spontaneous rupture and expectoration of pus and blood. His first attack of acute arthritis was twelve years ago. Both ankles and knees were involved. The duration of this illness was twelve days.

Present Illness.—On January 18th, 1916, the patient had an attack of sore throat. There was great swelling of the tonsils, with rupture and escape of blood and pus, from the left side of the throat, the patient thinks. On January 24th, five days after the onset, the patient first noticed pain in his right ankle and foot. There has been successive involvement of the other ankle, both knees and the right hip. He has had no chills or sweats. He thinks he has had slight fever. The joints have been painful.

Physical Examination.—The patient is a well developed man. There are one or two infected snags, and there is moderate pyorrhea. The tonsils are moderately hypertrophied,

and the mucous membranes of the throat are generally injected. The lymphatic glands of the neck are generally enlarged. A consultation from the Throat Service was asked for and an infection of the left antrum of Highmore was detected and drained.

The heart is normal.

There is tenderness in the left hip, both knees and ankles, with redness and swelling of the joints.

The urine is normal. Leucocytes are 17,500, with 82.5% neutrophiles. Blood culture is sterile. Wassermann test is negative.

The temperature rose to 102 the first two days, then became normal.

The tonsils were removed December 10th. The pyorrhea has been treated by the Dental Service.

The patient was discharged well on March 21st. He remained in the hospital some weeks after the joint symptoms cleared up at the request of the Dental Service.

CASE VII.

Miss B., aged 20, a nurse in training, complained of sore throat on February 11th and was admitted to the infirmary. She had been feeling poorly for about two days.

On admission her temperature was 102, pulse 120, respirations 20. She had acute follicular tonsillitis, temperature falling by lysis. It became normal on February 20th. It remained normal for four days. On the 25th of February the temperature rose to 99.6; on the 26th to 101.6. At this time she complained of abdominal pain and there was found localized rigidity and tenderness over McBurney's point. An operation was performed at once, and an acutely inflamed appendix was removed. The leucocytes at this time were 22,000. The temperature remained elevated between 100.4 and 103 until March 1st. It then ranged between 98.6 and 100 for several days. On the 28th of February the patient complained of aching in her limbs. The following morning, February 29th, both knees, shoulders, and some of the small joints of the hands were greatly swollen, red and extremely painful. The patient was given salicylates. The joint pains were controlled fairly promptly and the temperature came down, but the soreness and stiffness still remained to a certain extent. About March 10th, cultures were made from the right tonsillar region by Dr. E. C. Rosenow. Inoculation of two rabbits with this material produced an acute polyarthritis with multiple miliary abscesses in the myocardium. A staphylococcus was recovered from the tissues. The temperature began to rise on March 8th, and by March 13th it reached 102, pulse 100. Tonsillectomy, under local anesthesia, was performed on this date. The temperature rose to 103 the following day, and then for four days reached 102, gradually falling to a maximum of 99 on March 24th. Following the tonsillectomy the joint pains became worse for three days. About four days subsequent to tonsillectomy a mitral systolic murmur was first noted, and in spite of a return of the temperature to normal (though elevations to 99.4 or 99.6 occurred at intervals thereafter), the pulse rate remained high, varying for the most part between 90 and 110. After May 2nd, the temperature remained normal, the pulse was less frequent, and the patient was sent to her home for further convalescence.

CASE VIII.

Miss X., aged 20, had an attack of acute tonsillitis early in March. About two weeks following this, tonsillectomy was performed under local anesthesia. The fourth or fifth day following tonsillectomy, the patient first noticed pain and swelling in the knees and ankles, and complained of weakness and prostration. She was admitted to the hospital on April 3rd, 1916. Temperature, 101, pulse 96, respirations 28. Previous to operation the heart had been examined and found normal. On admission there were found moderate dilatation of the heart, both to the right and left, with a systolic murmur at the apex and accentuation of the pulmonic second sound. The temperature became normal on April 9th and remained so. The pulse rate also dropped to between 70 and 80, on this date and remained normal. The dilatation of the heart became less, but the evidence of a mitral insufficiency persisted.

CASE IX.

J. L., hospital No. A-777, was admitted to the North Medical Service of the Cincinnati General Hospital, February 2nd, 1916, complaining of vomiting of blood. The patient had several large hemorrhages, vomiting from one to two pints of blood at a time and the stools were tarry for several days. A diagnosis of gastric ulcer was made. He was given the Sippy treatment and made a complete recovery, the stools remaining free from blood after February 14th.

The patient was about to leave the ward when he developed an acute follicular tonsillitis on April 16th. April 21st the patient complained of pain in his left lower axilla, and a pleural friction rub was audible here. On the 25th blood was again found in the stools. On about April 24th the patient first complained of pain in his right knee which soon became swollen and hot and extremely painful on motion. The patient complained of some pain in others of the larger joints, but there was no definite redness or swelling. Blood cultures were sterile. The leucocytes were 28,000 on April 28th and there were 83 per cent of polynuclear neutrophiles.

The patient ran a continued fever until May 3rd. The temperature then became intermittent, and after May 12th there were rises to between 99 and 100. During June there was only an occasional rise.

The arthritis in the right knee did not yield to salicylates and a subacute arthritis persisted. In August the patient complained of pain in his spine in the lumbar region and x-ray examination showed a beginning hypertrophic arthritis. Owing to the experience with Miss B. and Miss X., it was decided, after consultation with the Throat service, not to enucleate the tonsils during the febrile stage of the disease. The operation was performed, however, in August. Within a week thereafter, the knee began to improve and within a month there was practically no pain in it. The patient was also completely relieved of pain in the back within three to four weeks. He has worn a plaster jacket which may account for the entire relief of pain in his back.

CASE X.

M. J., hospital No. A-5908, a colored widow, aged 45, was admitted to the North Medical Service of the Cincinnati General Hospital, September 2nd, 1916, complaining of "pain in the right side and back, misery in all the joints, fever and sweats."

The family history is negative.

Past History.—The patient had three miscarriages between the sixth and seventh months. She has had numerous attacks of sore throat since childhood. She never had acute arthritis.

Present Illness.—On August 17th, the patient complained of pain in the right side which radiated across the back. On August 22nd there was pain in the right shoulder and since then the pain has jumped from joint to joint. Fever and sweats began with the arthritis.

Physical Examination.—The patient is a short obese colored woman. There is marked pyorrhea alveolaris. The tonsils appear to be normal.

There is a systolic murmur at the apex. The aortic second sound is louder than the pulmonic second. The right ankle is swollen and hot. The knees are sensitive on pressure.

The urine is normal except for a few pus cells. Wassermann reaction is strongly positive. Gonococcus fixation test is negative. Blood culture is sterile.

The patient has an elevation of temperature to 100° F.

Tonsillectomy was advised by the Throat Service and performed on September 9th, 1916. The joint pains had been relieved by salicylates. The patient was entirely free from pain in her joints after September 15th. She was given mercury and iodide and was referred to the Dental Service for treatment of pyorrhea. The temperature continued to rise to 99.6 and it is probable that the alveolar infection accounted for this, as no other cause could be found. The patient was discharged September 26th improved.

CASE XI.

L. S., hospital No. 6434, a white male, single, aged 56, a peddler, was admitted to the North Medical Service of the Cincinnati General Hospital, December 6th, 1915, complaining of "rheumatism."

Family History.—The patient's mother died of "cancer of the stomach."

Past History.—The patient has had rheumatism about ten years. He had four or five attacks, each of them associated with fever, he thinks. He frequently catches cold from exposure to the weather.

Present Illness.—The present illness began November 29th, 1915, with pain and swelling in the left wrist. Later the right hand and right ankle were involved, followed by pain and swelling in both knees and finally in the left ankle. The patient has been unable to walk. The present attack is similar to the previous ones, though less severe.

Physical Examination.—The teeth are in very bad condition. The gums are retracted. The tonsils show no particular change. The throat is generally reddened.

The heart is normal.

Both ankles are swollen. The right one is red and painful. There are no tophi. Blood cultures are sterile. Wassermann is negative. Gonococcus fixation test is negative. Leucocytes 9,700, 65.5% neutrophiles.

On December 12th all of the carious and infected snags were removed.

The patient's temperature had been normal for three days and the joints were also painless. The patient was discharged on December 15th feeling well.

CASE XII.

R. K., hospital No. A-496, a white male, single, aged 52, a painter, was admitted to the North Medical Service of the Cincinnati General Hospital, January 21st, 1916, complaining of "rheumatism."

The family history is not of interest.

Past History.—The patient has had ten attacks of gonorrhea. For fifteen years he has averaged one attack a year of arthritis, involving the knees and ankles. The attacks have been of short duration, lasting three to seven days as a rule. The longest attack was of six weeks' duration and in this attack every large joint was involved. He was unable to work for three or four months after this attack. He drinks four to five glasses of beer and one glass of whisky daily. He gives no history of sore throat.

Present Illness.—The present illness began on January 18th, 1916, following four days' exposure to wet and cold. He complains of pain in both ankles and knees. He went to bed the day following the onset and came to the hospital two days later.

Physical Examination.—The patient is a well preserved man. The teeth are carious. There is marked pyorrhea alveolaris. The tonsils are not enlarged. Submental and posterior cervical glands are enlarged.

The heart is normal, except that the pulmonic second is louder than the aortic second.

There is swelling, redness and tenderness of the left knee and also tenderness and slight swelling of the left ankle.

The urine is normal. There are 7,800 leucocytes with no increase in the neutrophiles.

The temperature rose to 101 on admission, gradually falling to normal on the 5th day.

The teeth have been treated by the Dental Service. The joint symptoms have disappeared. The patient was discharged well on March 8th.

General conclusions may not be drawn from so small a series of cases. It is encouraging, however, to note that the fever subsided promptly after the operation in some of the patients. In Case II, Streptococcus viridans was isolated from the blood on two different occasions by Dr. Wherry, subsequent to the control of arthritis by salicylates, and fever and leucocytosis persisted. Within three or four days after tonsillectomy, the patient's temperature became normal and remained so. The patient is a market man, exposed to the weather, but he has remained well since his discharge from the hospital, about eleven months ago. This is our most encouraging case; for the evidence (excepting a bradycardia) pointed to a Streptococcus viridans endocarditis. Subsequent events practically disproved this and make it appear probable that microorganisms were gaining access to the circulation directly from the tonsils.

In conclusion, it seems to the writer advisable, in patients suffering with acute rheumatic fever, in whom evidence of tonsillar infection, acute or chronic, can be obtained, to remove the tonsils as soon as the joint pains can be controlled, provided the main conditions, as outlined in the body of the paper, can be fulfilled. Further experience alone can determine whether the so-called complications and recurrences of arthritis can be prevented in this manner.

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BACTERIEMIAS IN THE AGONAL PERIOD*

By J. W. Fredette, Pittsburgh, Pa.

THE presence of a terminal infection coincident with the lowered bodily resistance usually present for a variable period just preceding death has long been recognized, but much less is known of the relative frequency and type of the bacterial invasion. We are familiar with the fact that any condition which depresses or diminishes the physiologic activity and vitality of the body diminishes its ability to defend itself against bacterial invasion and so predisposes to infection. These changes are often so subtle that they escape detection and at times the vitality of the host is so lowered that no systemic reaction is manifested and the presence of a bacteriemia remains unsuspected. With these facts in mind the idea was suggested that routine blood cultures be taken in consecutive hospital cases immediately after death and irrespective of the cause of the fatal issue. It is to be remembered that the isolation of bacteria just after death gives no clue as to the time when the invasion took place. In some cases the bacteria found were an important factor in the disease, while in others they merely represented a late or agonal infection occurring at a time when the body defenses were at a low ebb.

The significance of terminal infections, particularly those which have occurred in the course of serious metabolic disturbances where no organic destruction of surface tissues has taken place, is difficult to estimate. It would appear that the bacterial invasion was spontaneous and arose in areas which naturally harbor these organisms. To say that these local tissues had suffered a lowering of vitality until their resistance was unable to withhold the migration of bacteria into the body, is only expressing in general terms our ignorance of the actual mode whereby mucosal surfaces serve as a protective barrier to bacterial invasion. The fact remains, however, that this is true, and, furthermore, that the conditions necessary for bacterial invasion do not require the death of the protective mucosal tissues. These conditions become particularly available when the general bodily state of metabolism is at a very low ebb. It was this state which we desired to study from the standpoint of the types of bacteria which would first take advantage of the lessened tissue resistance. The influence which these terminal infections have in hastening the death of the patient, we were unable to study closely; but we have no hesitation in saying that where an individual is harboring as virulent an organism in his blood stream as the streptococcus pyogenes, he is being seriously injured by it.

Great care was taken in the collection of the materials to exclude errors which might influence the interpretation of the results. The collecting outfit which we used consisted of tubes of plain serum broth, which was the culture medium used in all cases, sterile capillary pipettes, an alcohol lamp, scalpel, sterile

*From the Magee Pathological Laboratories, Mercy Hospital, Pittsburgh, Pa.

swabs, and tincture of iodine. These were kept in readiness and always available. Upon the death of a patient, the laboratory was immediately notified and the culture taken. The median basilic or cephalic vein was selected as the site for vein puncture. The skin was slightly drawn to one side and an incision made beside the vein, deep enough so that upon retraction of the skin the vein would stand out prominently between the everted edges of the incision. Tincture of iodine was then applied to the surface of the vein by means of a sterile swab. The tip of the sterile sealed capillary pipette was then broken, passed through the alcohol flame, and inserted into the vein. In the majority of cases, the capillary attraction and blood pressure within the vein was sufficient to fill the pipette with one or more c.c. of blood. The culture medium was immediately inoculated, and upon returning to the laboratory, placed in the incubator. The cultures were carefully watched for the appearance of growth and were without exception transferred to blood agar within twenty-four or thirty-six hours. Where a growth was observed, the organisms were plated, then picked for pure cultures, and subsequently transferred to their respective "sets." From the foregoing, it can be seen that the technic, while exceedingly simple, offers the least possible chance for contamination, inasmuch as the site of puncture does not come in contact with anything save the sterile swab saturated with iodine and the sterile capillary pipette. In only a few cases was any difficulty experienced in the removal of blood, and in these instances, it was due to the anemic state of the patient or to the small caliber of the blood vessels in children. The blood cultures were always taken within ten minutes following death and before the body had been in any way handled. This is a most important consideration; for even with the lapse of a very brief period after death, the manipulation of the body, such as occurs during its transference to the morgue, may cause the distribution of bacteria to sites other than those present during life. In all cases the cultural results here reported were obtained from materials collected from the body as it lay in bed.

From the following table it will be seen that out of the one hundred and nineteen cultures taken, forty-two were positive, and seventy-seven sterile. Furthermore, it will be noted that the organisms isolated belong to the types which are not uncommonly found in human infections of various tissues. While the number of positive findings may seem small, two factors should be considered: (1) the time postmortem, and (2) the type of case investigated. The time postmortem is important inasmuch as the possibility of a postmortem invasion of blood stream has been practically excluded. Postmortem invasion, however, is a very variable factor, as has been demonstrated by many investigators, and the possibility of such an occurrence is not to be overlooked. The type of case is also important, since in this series, consecutive cases were cultured irrespective of the cause of death. Then, too, the number of sterile cultures would indicate that the technic employed was a dependable one, since the ordinary contaminations were not present. The frequent sterility of postmortem blood cultures has also been the observation of other investigators. Strauch, in his study of two thousand postmortem blood cultures, found that in about one-half, or nine hun-

dred and ninety-eight times, the cultures were sterile. These were taken from the heart's blood at autopsy, and on an average of fifteen to sixteen hours after death.

TABLE I.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	BACTERIOLOGICAL RESULTS AT TIME OF DEATH.
1.	939	1676	F.	37	Fibromyomata of uterus	No growth
2.	943	1820	M.	7	Ac. append. and peritonitis	No growth
3.	950	1936	F.	37	Puerperal septicemia	B. acidi lactici
4.	951	1748	M.	66	Pneumonia—Cardiac dilatation	No growth
5.	968	1686	F.	48	Cholecystitis—Cholelithiasis	No growth
6.	972	1995	M.	39	Perforated gastric ulcer	Strept. salivarius
7.	999	2051	M.	69	Apoplexy—Chr. int. nephritis	No growth
8.	1013	2229	F.	24	Died during labor	No growth
9.	1017	2166	F.	28	Puerperal septicemia	No growth
10.	1021	2139	M.	16	Pneumococcic meningitis	Pneumococci
11.	1036	1996	M.	39	Sarcoma of lymph glands	Unidentified Gram-neg. bacillus
12.	1045	2019	M.	58	Carcinoma of prostate	No growth
13.	1065	2307	F.	35	Tubo-ovarian abscess	Staph. pyogenes aureus —Staph. albus
14.	1066	1804	M.	25	Lymphosarcomatosis	No growth
15.	1089	2131	M.	38	Ac. card. dilatation—Cirrhosis of liver	No growth
16.	1090	2295	M.	37	Chr. endocarditis—Coronary embolism	Strept. pyogenes—Staph. pyogenes aureus
17.	1098	2028	M.	36	Malaria—Sec. anemia	No growth
18.	1113	2349	M.	69	Cirrhosis of liver	No growth
19.	1117	2500	M.	44	Lobar pneumonia	No growth
20.	1121	2484	M.	24	Chronic nephritis	No growth
21.	1158	2541	M.	30	Chr. int. nephritis	No growth
22.	1175	2567	M.	21	Rupture of small bowel	No growth
23.	1193	2864	M.	65	Chr. endocarditis	No growth
24.	1229	3050	M.	31	Bronchopneumonia	Staph. pyogenes aureus
25.	1247	3045	M.	25	Typhoid fever	B. typhosus
26.	1270	2921	M.	49	Miliary tuberculosis	No growth
27.	1269	3109	M.	10	Supp. otitis media and meningitis	No growth
28.	1281	3135	F.	42	Pyosalpinx—Peritonitis	No growth
29.	1282	2968	M.		Diffuse peritonitis	Strept. viridans — Micrococcus?
30.	1332	2963	M.	36	Myelogenous leukemia	Strept. pyogenes
31.	1328	3342	M.	16	Hydrophobia	No growth
32.	1333	3381	M.	40	Lobar pneumonia	No growth
33.	1348	3391	M.	33	Delirium tremens	No growth
34.	1537	3395	M.	52	Ac. miliary tbc.	Strept. mitis—B. acidi lactici
35.	1371	3320	M.	53	Chr. myocarditis.	No growth
36.	1373	3479	F.	7 mos.	Intestinal obstruction	No growth
37.	1390	3420	M.	33	Pneumonia and tuberculosis	Pneumococcus
38.	1402	3374	F.	36	Hyperthyroidism—Thyroidectomy	No growth
39.	1416	3551	M.	66	Lobar pneumonia	Strept. salivarius
40.	4	3753	F.	45	Hypernephroma of ureter	No growth
41.	27	3874	M.	32	Fracture pelvis—Traumatic ileus	No growth

TABLE I.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	BACTERIOLOGICAL RESULTS AT TIME OF DEATH.
42.	28	3750	F.	42	Fibroid uterus — Cholelithiasis	No growth
43.	45	3146	F.	57	Pernicious anemia	Strept. anginosus
44.	47	3931	M.	54	Lobar pneumonia	Strept. salivarius—Strept. mucosus
45.	68	3940	M.	33	Lobar pneumonia	Pneumococcus
46.	69	3856	F.	47	Cholelithiasis	No growth
47.	70	3713	F.	18	Chr. arthritis—Ac. nephritis	No growth
48.	73	3956	M.	64	Lobar pneumonia	Pneumococcus — Staph. albus
49.	98	3897	M.	60	Ac. retention—Stricture of urethra	No growth
50.	102	4091	F.	84	Cerebral hemorrhage	No growth
51.	103	4092	M.	5	Hernia	No growth
52.	116	4207	M.		Lobar pneumonia	Pneumococcus
53.	127	3672	M.	47	Nephritis and pneumonia	Pneumococcus
54.	128	4213	F.	76	Strangulated hernia—Peritonitis	Strept. mitis—B. pseudodiphtheria
55.	149	3717	M.	40	Chr. interstitial nephritis	No growth
56.	158	2311	F.	47	Pyosalpinx and pelvic abscess	No growth
57.	181	4485	M.	70	Lobar pneumonia	Pneumococcus
58.	184	4535	M.	55	Lobar pneumonia	Pneumococcus
59.	196	4405	F.	63	Complete prolapse of uterus	No growth
60.	213	4261	F.	51	Systocele—Rectocele	No growth
61.	214	4688	M.	25	Pulmonary tuberculosis	No growth
62.	216	4617	M.	34	Cerebrospinal lues	No growth
63.	231	4750	M.	35	Tumor of base of tongue	No growth
64.	257	4836	M.	43	General peritonitis	Strept. pyogenes
65.	258	4762	F.	29	Puerperal septicemia	Strept. pyogenes
66.	285	4885	M.	20	Septicemia	Strept. infrequens
67.	300	5086	M.	6	General peritonitis	No growth
68.	303	5032	M.	44	Lobar pneumonia	No growth
69.	306	5072	M.	53	Lobar pneumonia	No growth
70.	312	4293	M.	24	Broken back	No growth
71.	340	4866	M.	46	Appendiceal abscess — General peritonitis	Strept. pyogenes
72.	347	5315	M.	35	Lobar pneumonia	No growth
73.	368	5390	F.	2	Fecal impaction	Staph. pyogenes aureus
74.	369	5288	M.	41	Lobar pneumonia	Pneumococcus — Strept. pyogenes
75.	392	5253	M.	26	Pneumonia	Pneumococcus — Strept. pyogenes
76.	423	5491	M.	32	Cholecystitis—Appendicitis	Strept. pyogenes
77.	427	5493	M.	67	Hypertrophy of prostate	No growth
78.	448	5304	M.	72	Papilloma of bladder	Strept. equi
79.	454	5531	M.	67	Carcinoma of stomach	Strept. pyogenes
80.	459	4168	M.	44	Amebic dysentery	Strept. mitis
81.	470	5674	M.		Lobar pneumonia	Pneumococcus
82.	481	5702	M.	30	Lobar pneumonia — Pyopneumothorax	No growth
83.	482	5268	M.	5	Hemolytic anemia	No growth
84.	483	5550	M.	57	Septicemia following infected thumb	Staph. pyogenes aureus
85.	489	5910	F.	19	Puerperal septicemia	Strept. pyogenes
86.	498	5300	M.	48	Lymphosarcoma — Abscess of lung	Strept. hemolyticus Strept. viridans
87.	500	5946	M.	54	Fatty degeneration of heart	No growth

TABLE I.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	BACTERIOLOGICAL RESULTS AT TIME OF DEATH.
88.	508	5947	M.	36	Chronic nephritis	No growth
89.	530	6094	M.	45	Carcinoma of stomach	No growth
90.	550	6289	M.	60	Pneumonia	Pneumococcus
91.	552	5766	F.	38	Heart disease	No growth
92.	562	6339	M.	19	Ruptured liver	No growth
93.	573	6363	M.	29	Acute alcoholism	No growth
94.	576	6283	M.	53	Diabetic gangrene of foot	No growth
95.	589	6257	F.	46	Dermoid cyst of ovary	No growth
96.	607	6441	M.	61	Aneurysm of aorta	No growth
97.	608	6156	M.	33	Alcoholism—convulsions	No growth
98.	666	6195	M.	40	Amebic dysentery	No growth
99.	669	6121	F.	67	Chronic pancreatitis	Staph. pyogenes aureus
100.	671	277	M.	72	Heart, kidney, and vascular disease	No growth
101.	690	6199	M.	44	Brain tumor	Pneumococcus
102.	709	511	M.	47	Pneumonia?	No growth
103.	714	2214	M.	21	Fracture 12th dorsal vertebra	No growth
104.	736	489	M.	26	Peritonitis—Ventral hernia	No growth
105.	737	602	F.	48	Intestinal obstruction	No growth
106.	742	205	F.	35	Tuberculosis of spine	No growth
107.	743	189	F.	58	Acute suppurative cellulitis of hand	Strept. salivarius—Staph. albus
108.	755	481	M.	36	Multiple burns	No growth
109.	762	6206	F.	39	Peritonitis following appendectomy	No growth
110.	763	194	M.	49	Carcinoma ampulla of Vater	No growth
111.	765	6540	M.	37	Abscess of liver	No growth
112.	770	639	F.	5	Meningitis	No growth
113.	772	658	F.	13	Peritonitis following appendectomy	No growth
114.	773	887	M.		Dislocation of hip	No growth
115.	774	905	M.	40	Pulmonary tuberculosis	Pneumococcus
116.	782	865	F.	62	Acute dilatation of heart	No growth
117.	784	956	M.		Heat stroke—Alcoholism	No growth
118.	785	925	M.	4 mos.	Plastic operation—Harelip	No growth
119.	791	792	M.	45	Pneumonia?	No growth

From the table it will be noted that of the forty-two positive cultures, thirty-one showed the presence of one organism, and eleven showed two organisms.

The frequency of the various types of organisms was as follows: streptococcus hemolyticus alone nine times, in all thirteen times; streptococcus viridans alone four times, in all ten times; pneumococcus alone eleven times, in all fourteen times; staphylococcus pyogenes aureus alone four times, in all six times. Staphylococcus albus was not found alone, but with other organisms, three times; B. acidi lactici alone once, in all twice; B. typhosus and an unidentified Gram-negative bacillus once. From the results obtained, it will be seen that the streptococcus was the most frequent organism isolated and the pneumococcus next. This was also the observation of Strauch whose results were as follows: of the one thousand and two positive cultures, streptococci were found alone four hundred and sixty times, in all five hundred and forty-eight times; pneumococci alone one hundred and fifty-five times, in all one hundred and ninety-seven times;

colon bacilli alone one hundred and thirty-two times, in all one hundred and thirty-seven times; staphylococci alone ninety-five times, in all one hundred and thirty-eight times; paratyphoid bacilli alone fourteen times, in all sixteen times; pneumo-bacilli (Friedlander) alone ten times, in all fourteen times; bacilli emphysematous (B. welchii) alone twice. Of the two thousand cases examined, the blood, therefore, was positive in 50.1 per cent; and in eight hundred and eighty-one times there was one type of bacterium, that is, 87.9 per cent of all positive findings; mixed infections of two types occurred one hundred and fourteen times, that is, 11.4 per cent; and a mixed infection of three types occurred only seven times, or 0.4 per cent of all cases.

As we have observed, and this would undoubtedly be substantiated in a larger series of cases, the streptococcus is the most frequent terminal invader. The occurrence of streptococcus pyogenes in cases 74 and 75, with the pneumococcus, is extremely interesting, and further careful study, in fatal cases of pneumonia, might show that the presence of such a coincident infection is relatively frequent.

In the course of the compilation of this data it occurred to us that a comparison of the bacteriological findings of the blood before and after death would be particularly interesting, in view of the fact that similar results would confirm the provisional antemortem bacteriological diagnosis, while the finding of additional organisms, would more firmly establish the more or less theoretical supposition of "agonal" or terminal infection. In reviewing the bacteriological reports extending over the period of investigation it was found that only fourteen antemortem blood cultures had been requested upon the one hundred and nineteen cases here studied. This is strikingly significant when we consider that in this brief series forty-two positive cultures were found. It further emphasizes that either the bacteriemias existed when the clinical manifestations of them were apparently not of sufficient import to justify the requisition of a blood culture, or that the infection had occurred very late in the progress of the disease when its presence had no clinical bearing. Unquestionably, bacteriemias, and oftentimes fatal ones, do exist where they are unsuspected. This, I believe, would be found particularly true in surgical cases where there is often a post-operative rise in temperature and an absence of the other generally recognized signs of blood stream invasion. However, it is not our purpose to discuss those bacteriemias existing for a considerable time before death.

We have long been familiar with the fact that overwork, previous infection, malnutrition, diet, intoxication, exposure, and trauma frequently lower the bodily resistance and predispose to infection. Why, then, should we not assume that morbid conditions in general, particularly immediately before death and when the bodily vitality is at its lowest ebb, offer the most favorable opportunity for a general bacterial invasion? This supposition, in order to be conclusive, must of course be proved by a greater number of cases than have here been presented, but it is earnestly hoped that the work may be continued. The securing of frequent antemortem cultures would be the most logical way in which to check the postmortem results.

The following table illustrates our results before and after death:

TABLE II.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	A. M. DATE.	RESULT.	P. M. DATE.	RESULT.
1.	1008	2166	F.		Puerperal septicemia	Oct. 5-15	Strep. pyogenes	Oct. 7-15	No growth
2.	1017 1057 1090	2295	M.	37	Chr. endocarditis—Coronary embolism	Oct. 13-15	Staph. pyogenes aureus	Oct. 17-15	Strept. pyogenes Staph. pyogenes aureus
3.	1209 1270	2921	M.	49	Miliary tuberculosis	Nov. 17-15	No growth	Nov. 28-15	No growth
4.	1227 1229	3050	M.	31	Bronchopneumonia	Nov. 20-15	No growth	Nov. 21-15	Staph. pyogenes aureus
5.	900 1138 1282	2968	M.	31	Diffuse peritonitis—Acute gangrenous appendicitis	Sept. 10-15 Oct. 31-15 Dec. 7-15	B. lactis aerogenes No growth No growth	Nov. 29-15 Dec. 8-15	Strept. viridans micrococcus (?) No growth
6.	1322 1328	3342	M.	16	Hydrophobia	Feb. 29-16 Mar. 11-16	Strep. infrequens No growth	Mar. 3-16	Streptococcus infrequens
7.	275	4885	M.	20	Septic arthritis	Mar. 15-16	No growth	Mar. 18-16	Strept. pyogenes
8.	316 333 340	4866	M.	46	Appendiceal abscess	Mar. 18-16	No growth		
9.	335 438 482	5268	M.	5	Hemolytic anemia (Aplastic)	Apr. 11-16	No growth	Apr. 22-16	No growth
10.	377 392	5253	M.	36	Lobar pneumonia	Mar. 29-16	No growth	Apr. 1-16	Pneumococcus Strept. pyogenes
11.	456 483	5550	M.	57	Septicemia (infected thumb)	Apr. 16-16	Staph. pyogenes aureus	Apr. 22-16	Staph. pyogenes aureus
12.	488 489	5910	F.	19	Puerperal septicemia	Apr. 25-16 (4 hrs. A. M.)	B. acidi lactici	Apr. 25-16	Strept. pyogenes
13.	625 643 651	6540	M.	37	Abscess of liver	June 1-16 June 7-16 June 9-16	No growth No growth No growth	July 3-16	No growth
14.	765 739 772	658	M.	13	Peritonitis following appendectomy	July 3-16	No growth	July 12-16	No growth

TABLE III.

NO.	BACT. NO.	HOSP. NO.	BACTERIOLOGICAL RESULT IMMED. P. M.	AUTOPSY NO.	TIME OF AUTOPSY	PATHOLOGICAL DIAGNOSIS.	BACTERIOLOGICAL DIAGNOSIS.
1.	950	1936	B. acidi lactici	A-34-15	2 hrs.	Rupture of uterus and peritonitis	B. acidi lactici
2.	1066	1804	No growth	A-38-15	10 hrs.	Lymphosarcomatosis	No growth
3.	1090	2295	Strept. pyogenes Staph. pyogenes aureus	A-40-15	12 hrs.	Heart disease and coronary embolism	Strept. pyogenes Staph. pyogenes aureus
4.	1123	2484	No growth	A-42-15	4 hrs.	Acute Bright's disease	B. acidi lactici
5.	1229	3050	Staph. pyogenes aureus	A-47-15	6 hrs.	Lobar pneumonia	Staph. pyogenes aureus
6.	1247	3045	B. typhosus	A-48-15	1 hr.	Typhoid fever with hemorrhage	B. typhosus
7.	1270	2921	No growth	A-50-15	11 hrs.	Miliary tuberculosis	No growth
8.	1332	2963	Strept. pyogenes	A-52-15	4 hrs.	Chloroma	Strept. pyogenes
9.	1328	3342	No growth	A-53-15	2 hrs.	Hydrophobia and acute enteritis	No growth
10.	1333	3381	No growth	A-55-15	2 hrs.	Hypostatic pneumonia	No growth
11.	1348	3391	No growth	A-56-15	6 hrs.	Infected clavus of toe with septicemia	No growth
12.	45	3146	Strept. anginosus	A-1-16	1 hr.	Pernicious anemia with cholecystitis	Strept. anginosus
13.	224	4691	No growth	A-10-16	2½ hrs.	Heart, kidney, and arterial disease	No growth
14.	231	4750	No growth	A-11-16	12 hrs.	Sarcoma of tonsil	No growth
15.	340	4866	Strept. pyogenes	A-14-16	3 hrs.	Appendiceal abscess with gen. peritonitis	Strept. pyogenes
16.	459	4168	Strept. mitis	A-19-16	10 hrs.	Amebic dysentery with chronic parenchymatous nephritis	Strept. mitis B. lactis aerogenes
17.	530	6094	No growth	A-23-16	12 hrs.	Cancer of stomach	Strept. fecalis B. lactis aerogenes
18.	671	277	No growth	A-26-16	3 hrs.	Heart, kidney, and arterial disease	No growth
19.	742	205	No growth	A-29-16	16 hrs.	Tuberculosis of spine	No growth

It will be noted that in cases 2, 4, 5, 8 and 12, additional or different organisms were isolated immediately after death. Case 12, presented a very interesting finding, inasmuch as four hours before death B. acidi lactici was isolated, whereas immediately postmortem, streptococcus pyogenes was found. These cultures were both taken from the same median cephalic vein, four hours apart, and yet each yielded a pure culture. In case 1, streptococcus pyogenes was isolated in the antemortem culture but was not obtained postmortem. In numbers 3, 6, 7, 9, 11, 13 and 14, the same results were obtained both antemortem and postmortem.

The value of late postmortem bacteriological findings, as obtained at autopsy, and particularly in those cases autopsied some time after death, has long been a subject of discussion. It was, therefore, believed that a comparison of cultures obtained immediately postmortem with those obtained later at autopsy would be significant.

The comparison suggested will be noted in the following table. The number, while too few to be conclusive, at least points out the possibilities of future investigations along this line. The cases, nineteen in number, were autopsied one to sixteen hours after death. Of that number, sixteen showed the same results at autopsy, while three showed further bacterial invasion. The postmortem bacterial invaders were B. acidi lactici, B. lactis aerogenes, and streptococcus fecalis.

CONCLUSIONS.

1. Routine blood cultures taken immediately after death reveal the presence of an unsuspected bacteriemia in about one-third of all fatal cases.

2. Streptococci are the most frequent terminal bacterial invaders of the blood stream. The pneumococcus can be isolated in practically all cases of lobar pneumonia dying before the tenth day of the disease.

3. Bacteriological findings at autopsy within a few hours after death, though fairly reliable in demonstrating the presence of organisms existing at the time of death, do not exclude the possibility of postmortem invasion.

4. The taking of frequent antemortem, immediate postmortem, and autopsy cultures is to be encouraged. Contamination may be obviated by a simple technic.

5. In the absence of adequate autopsy material, the routine taking of immediate postmortem cultures will furnish valuable information as regards the essential and terminal bacteriemias.

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THE DIAGNOSIS OF GASTRIC ULCER

By John W. Shuman, M.D., Sioux City, Iowa.

WHEN a "stomach complainer" consults, we should take a careful and complete history of that patient's health and record all the facts. If in the history such subjective symptoms as hyperacidity (sour eructations), hunger-pain, one to two hours after eating, which is relieved by the taking of food, and hypersecretion are stated, an ulcer of the gastro-duodenal area is to be differentially diagnosed from chronic appendicitis, cholecystitis, pancreatitis and oftimes other diseases.

If a thorough, painstaking, head-to-foot physical examination is made on this ulcer suspect and there is an absence of any visceral pathology demonstrable to inspection, percussion, and palpation, excepting a hypersensitive area or two in the abdomen, one or two tests should be made to exclude active ulceration. A general physical examination is extremely necessary. The finding of pus pockets in tonsils, teeth or elsewhere in a positive ulcer case demands eradication as a rational therapeutic procedure.

For the finding for or against active ulceration nothing is easier than the use of the No. 14 braided-silk cord and lead ball test. I have been using this test for a little over five years, first reporting it three years ago.[1]

The finding of a blood stain on the cord denotes bleeding from a raw surface and gives in a measure its location. The clean cord finds against ulceration.

I have checked this test against every possible source of error and have found it correct. In all cases operated upon in which the cord test was "positive" the surgeon found an ulcer. In one case, the cord showed two blood stains; two separate and distinct ulcers were found at autopsy.

The use of the duodenal tube is quite necessary in many cases in which one is attempting differential gastric diagnostic work. By using it carefully and observing, one can tell when the bulb passes into the duodenum. If blood (bright red shreds) is aspirated prior to the bulb passing out of the pylorus, ulceration on the gastric side is evident; if after passing the pylorus blood is aspirated, an ulcer on the duodenal side of the pylorus is just as evident. By securing the duodenal secretions and examining one can also tell something of the biliary and pancreatic function.

For differential and confirmatory diagnosis the roentgen ray is indispensable. I do not believe any one test sufficient to establish a diagnosis. All methods of diagnostic technic should be used in a given case. When they are not used it is either because of laziness, inefficiency or uninterestedness. The more careful, thorough and conscientious our work, the fewer mistaken diagnoses will result.

In the roentgen ray examinations of the hollow viscera of the abdomen, I follow the plan of using two to three ounces of barium sulphate and twenty-four to thirty-two ounces of buttermilk for the opaque meal. Six ounces of

[1]St. Paul Med. Jour., April, 1914, later Jour. Lancet, Aug., 1916, and Kan. City Med. Herald.

barium sulphate and thirty-two ounces of "stale" buttermilk (98° F.) are used for the opaque enema. Examination is made with the roentgenoscope while the opaque matter is passing into the stomach or colon. If any abnormality is noted an "immediate" exposure is made for record. At six hours the stomach is again examined. Ofttimes several exposures are necessary before a correct interpretation can be attempted.

The roentgen ray findings upon which I place the highest value are, first, a diverticulum; second, a permanent incisura opposite the site of the ulcer; third, hyperperistalsis (duodenal ulcer); fourth, a large six-hour residue (pylorospasm); and fifth, a deformed cap or duodenum, which shows constantly in a series of plates (duodenal ulcer). Hyperperistalsis and a large six-hour residue are highly suggestive, and if in addition we have a permanent incisura opposite the cite of the ulcer we can be certain of the presence of ulcer. A diverticulum is practically diagnostic of penetrating ulcer. If a deformed cap or duodenum is present I feel safe in diagnosing duodenal ulceration from the roentgenoscopic examination.

Here are two cases reported in full for comparative study in differential gastric diagnostic technic. Both complained of "stomach trouble," both were referred for "treatment of gastric ulcer." One had ulcer of the stomach, the other chronic appendicitis.

Case 1.—Mrs. S. Married, age 40. Was referred Feb. 5th, 1916, complaining, "stomach has been hurting a long time."

Family history, unimportant. Previous diseases: scarlet fever at 25; at 36 confined to bed for four weeks with "inflammation of the left lung." At 38 a part of the left nipple was excised; diagnosed "Paget's disease."

Present illness; began one and one-half years ago with bloating after eating; pain severe two to three hours after eating; vomiting relieved gastric hypertension. Weight then 125 pounds, now 109¾. At present, "aches through abdomen" and is stiff in limbs and back; stomach is sour; "baking soda relieves the stomach symptoms." Constipated. Menses very irregular.

Nocturia 1 2 Sleeps fair.

Day nocturia 4 5

Physical examination; T. 99.2, P. 119, R. 25. Hemanalysis; Hgb. 75% W.B.C.8,300. R.B.C.3,800,000. Wassermann negative. Urinalysis: straw, cloudy, acid, 1026, phosphates positive. Albumin, sugar and microscopic examination negative.

Mouth; severe pyorrhea of all but four lower front teeth. Tonsils submerged and diseased. Chest negative for active disease findings. Abdomen; a tender movable, walnut-sized mass just above and a little to the right of the navel (thought to be pylorus). Vaginal and rectal examination, negative. Extremities; second degree varicosities of left lower limb. Stool—occult blood positive (meat-free diet). Braided-silk cord test positive. Fig. 1 shows this cord with a two and one-half inch blood stain upon it, just a short way from the bile stain. roentgen ray examination showed a fish-hook stomach and a deformed cap which was constant through a series of (7) exposures. (See Fig. 2.)

Operation was advised on account of the duration and severity of symptoms

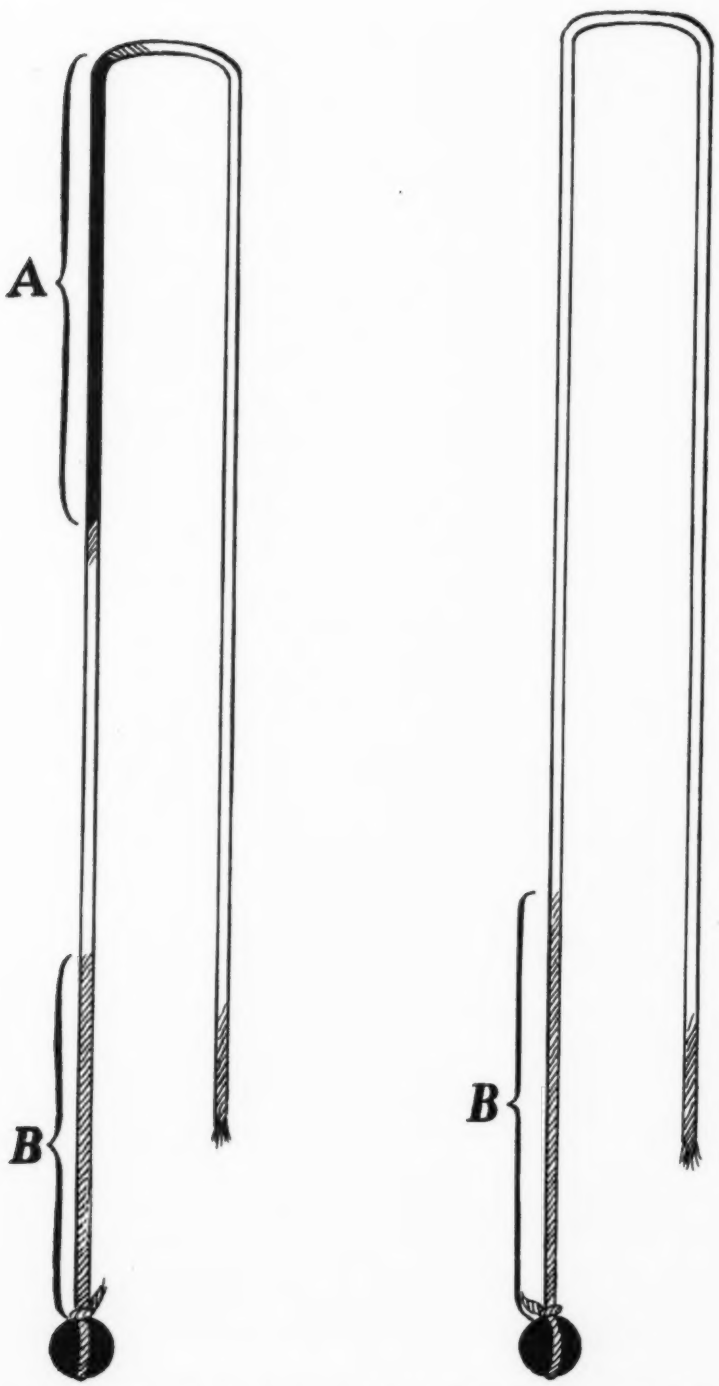

Fig. 1.—Actual size of cord and ball used in Case 1. The length of the string was 30 inches. *A* shows the two and one-half inch blood stain (dark red) and *B*, the bile (yellow) stain with a clear space intervening. Compare with the negative cord on the right of Case 2.

Fig. 2.—Case 1. Position prone; time, 30 minutes. Reading; enlarged, fish-hook shaped stomach. (*A*) Pylorus. (*B*) Deformed duodenal cap which was constant throughout examination. Compare with Fig. 3—a normal cap.

Fig. 3.—Case 2. Position prone; time, first meal 24 hours, second meal 10 min. Reading; normal stomach and cap. (*A*) Gastric spasm which did not remain constant during examination; diagnosed as reflex caused by (*B*) a clubbed appendix which did not empty for ten days when all other organs were clear of barium in forty-eight hours.

and the history of malignancy of the left breast, but the patient refused. Her diseased tonsils and teeth were removed. She was put to bed and the non-irritation treatment for pyloric ulcer administered. At the end of six weeks she had gained six pounds in weight and lost all feeling of discomfort and pain, the tender walnut-sized lump in the pyloric region had also disappeared. At the end of six months (Aug. 5th, 1916), she had gained ten pounds and was "feeling the best she had for three years." The time is too short to say more of the "cure."

Case 2.—Mr. B., single, age 23, clerk, was referred June 2nd, 1916, complaining of "stomachache." Family history; one brother dead at 31, "gall stones" (unoperated). Previous diseases; left sided pleurisy off and on for 2 to 3 years. One and one-half years ago operated upon and a pea-sized calculi was removed from the left ureter; since then no pleurisy. Present illness; began four weeks ago with almost constant nausea. No appetite for three or four days. Since then no trouble until four days ago when he suffered a similar attack. Soon after eating, his stomach hurts and continues to pain until the next meal. Has lost seven pounds in six weeks.

Physical examination; T.99. P.85. R.16. Hemanalysis; Hgb. 85%. W.B.C.-9,000. R.B.C. 4,500,000. Urine; straw, clear acid, 1020. Phosphates positive, albumin, sugar and microscopic tests negative. Stool; occult blood, negative. Head and chest negative. Upper abdomen negative. Tender McBurney point. Rectal and genitalia examination negative. Roentgen reading; a clubbed appendix causing a reflex spasm of the stomach. (See Fig. 3.) Operative findings confirmed this diagnosis as did the subsequent history, i. e., uneventful recovery and no gastric disturbance since.

Many diseases can simulate gastric disease until a thorough examination is made.

LABORATORY METHODS

THE DETECTION OF SEMEN

By Victor C. Vaughan, M.D., Ann Arbor, Mich.

IT is understood that in rape there may not be either immissio penis or emissio seminis though both may happen and either may occur without the other. In women not virgins defloration cannot occur because it has already happened, but in all cases the investigation should include a careful search for semen which may be deposited in the vagina, between the labia, on other parts of the person, on the clothing, on the bed, floor and ground. In case death has resulted the search for semen should not be confined to the locations mentioned but should extend to the uterus and Fallopian tubes. Unfortunately the polluted victim frequently washes herself and possibly her clothing before an examination can be made. In favorable cases spermatozoa may be found hours and days after the act. Mashka found spermatozoa in the vagina of a girl twelve hours after she was raped, and the same author quotes Downe who found spermatozoa in the vagina of a woman who had been in the hospital for some days, and Bayard, who was successful in his search three days after copulation. Bossi states that elements of the semen may be found in the vagina from twelve to seventeeen days after coitus, while Hausmann was unable to find them after thirty-six hours. Haberda states that when the genitals are healthy the spermatozoa may, within a few hours, find their way into the Fallopian tubes. Experimentally this has been found to happen in some of the lower animals within two hours, and there seems to be no reason why it should not occur with equal promptitude in woman. When the secretions are healthy, living spermatozoa may be found a long time after the last coitus. Dührssen, in an operation for pyosalpinx, found motile spermatozoa in the less affected tube, although the woman had been in the hospital nine days and three and one-half weeks had passed since the last coitus. Spermatozoa may retain their vitality in the dead bodies of animals for some days and Ivanoff found that they may fecundate when taken from the vesicles twenty-four hours after death. In two cases in which death occurred during or immediately after coitus, Haberda found spermatozoa in the vagina, but after a most careful search failed to find any in the uterus or Fallopian tubes; however, this author states that Zweifel reports an instance in which death occurred during coitus and sixteen hours later motile spermatozoa were found in the Fallopian tubes, but these may have come from a previous copulation. It seems from these statements that the finding of spermatozoa in the uterus and tubes does not prove conclusively that coitus has immediately preceded death unless it can be shown that there had been no recent, but prior, indulgence in the sexual act.

The external genitals should be carefully examined for seminal stains. With only vulval penetration or even when the connection does not go so far

as this there may be emissio seminis. We must also recognize the fact that some of the old sexually exhausted men who commit rape on children select their victims because they are unable to copulate with adult women, and some of these blennorrhagic individuals have no spermatozoa in their semen. In such instances the proof that a stain is seminal cannot be positive and complete.

Some authorities have placed considerable stress upon the contour, gray coloration and odor of seminal stains especially when deposited upon white cloth, but it is sufficient to state that in none of these appearances is there anything sufficiently characteristic of seminal stains to enable the investigator to arrive at a positive conclusion, and the statement of the expert must be founded upon incontrovertible fact and not upon mere belief or suspicion. The only conclusive evidence that a stain is of seminal origin depends upon the finding of spermatozoa. Other morphological elements may or may not be present, but spermatozoa only characterize seminal fluid and unless their presence be demonstrated the evidence must be considered as negative. Epithelial cells, so-called seminal granules and spermatic crystals may or may not be present in seminal fluid and some of them certainly are found in stains from other sources. No one should attempt to decide concerning a suspected seminal stain unless he is thoroughly familiar with the appearance of spermatozoa in both fresh and dried specimens. In freshly ejaculated semen and in the mucus of the normal female genitals, they may retain their motility for a long time and in dried stains they may preserve their form and may be easily recognized under the microscope even after many years. If the stain be upon cloth, leather, wood or metal and if it be of sufficient thickness it is better to detach a bit of it from the object to which it is adherent and rub this up with a few drops of physiologic salt solution and examine it under the microscope. If the stain cannot be detached from the substance to which it adheres and which generally consists of some article of clothing, a small bit of the cloth carrying the stain should be cut out, placed in a watch glass, treated with a few drops of salt solution, covered so as to prevent evaporation and to protect it from dust and allowed to stand for two or more hours. Usually this results in the formation of a milky fluid, a drop of which may be subjected to direct microscopical examination. Another method is to place a bit of the cloth carrying the stain on an object glass, add a few drops of water or physiologic salt solution and with needles tear the fibres of the cloth apart; then, cover with a thin glass and examine with the microscope. When the stain is very old it is well to render the salt solution used for maceration feebly alkaline with ammonia. When a more active solvent is needed, as in the case of the presence of a large amount of dried mucus, a dilute solution of potassium hydroxid or a five per cent solution of acetic acid may be employed. Spermatozoa are fairly resistent to both acids and alkalis. Roussin recommends that the stain be soaked in a solution made up of 1 part of iodin, 4 parts of potassium iodid and 100 parts of water, and Unger advises that the spermatozoa be stained with a solution of methyl green (0.3 g. of methyl green to 100 of water, acidified with 6 drops of hydrochloric acid).

It is not safe to depend upon granules that appear to be the heads of

spermatozoa, because there are too many things that may be mistaken for these. More or less complete cells with both head and tail should be found before a positive conclusion is reached.

When the mucous secretion of the genitals is semi-fluid it is sufficient to take a drop of it and make with this a thin smear and examine directly with the microscope. When the mucus is dry a few drops of a salt solution may be added in order to dilute and dissolve it.

Florence has discovered a method of preparing from seminal stains crystals which he believes to be characteristic of spermatic fluid. The reagent employed consists of the following ingredients: Potassium iodid, 1.6 g.; iodin, 2.54 g., and water 30 g. This reagent should be cooled by surrounding it with ice water for some hours or by keeping it in an ice box for twenty-four hours before the test is made. The examination is carried out as follows: A bit of the suspected stain in a drop of the maceration obtained from it is placed on a glass slide, then a drop of the reagent is placed sufficiently near so that the two when covered with the thin glass gradually flow together. The observer looking through the microscope will see the crystals form as the two fluids come in contact. These crystals are brownish, rhombic prisms closely resembling those of hematin, although there may be many atypical forms. They are soluble in water, alcohol, acids and fixed alkalis. Individual crystals may be large enough to be seen by the unaided eye. They are not permanent, though according to Richter they may be preserved for a time, at least, by the addition of a few drops of a solution of iodic acid and enclosing in a cell of wax. According to Florence the formation of these crystals is characteristic not only of seminal stains, but of those from man, inasmuch as he finds that they are not found in the spermatic fluid of other animals. He believes that the presence of these crystals, even when spermatozoa cannot be detected is evidence that the stain is made by the spermatic fluid of man. This is important if true, but Richter holds that the crystals may be obtained from many substances, such as decomposing egg-white, egg-yolk, pus, vaginal and uterine mucus, the semen of the lower animals, certain vegetable substances and butter, and Gumrecht suggests, with great probability, that they are lecithin compounds and that when putrefaction advances far enough to break up the lecithin molecule even human semen does not give them. Haberda states that in some badly decomposed vaginal secretion taken from a corpse he found many well formed spermatozoa, but could not obtain the Florence crystals although his reagent gave good results with other seminal stains and with the semen of a man with azöospermia. This shows that the proposition of Binder that the Florence test be used as a preliminary one and that when it fails the search for spermatozoa need not be continued is not wise. Haberda, however, thinks that the test may be of service in picking out the one stain among many most likely to show spermatozoa. Dvornitschenko finds that the Florence test is not possessed of positive value, that there are substances that do not come from seminal fluid that yield the crystals and that when the reaction is negative spermatozoa may be found. He concludes that the substance in seminal fluid which takes part in the formation of the crystals is not a constant constituent of the secretion. In short, this

author agrees with many others in finding that Florence's reaction is not possessed of absolute value, and that it is of service only as a confirmatory or supplementary test in the identification and recognition of spermatozoa. It seems, therefore, that we must hold to the old view that the detection of spermatozoa is the only sure proof that a stain is made by semen and the finding of these cells needs no confirmatory or supplementary test.

Staining spermatozoa in order to bring them more distinctly to view has been recommended, and we have already referred to the methods of Roussin and Unger. Florence uses crocein, and Grigorgew recommends the following method: A bit of the cloth carrying the stain is placed in concentrated sulphuric acid and left until the fibre is quite well destroyed. This may require seventy-two hours, but as a rule the partly disintegrated fiber, after four, eight or twelve hours, still saturated with acid, is placed on a glass slide, covered and examined under a magnification of 600 diameters. The heads of the spermatozoa are oval or pear shaped and brown in color, and show a lighter point or vacuole. The tail is not colored so deeply. The method is not applicable to stains on brown or black cloth. It seems that some years ago Vogel recommended a somewhat similar method; the bit of cloth is moistened with water, placed on the glass slide, two drops of sulphuric acid and, after two minutes, the same amount of tincture of iodin is added, after which the examination is made. It is claimed that the sulphuric acid method is of special value when the stain is found upon a much soiled piece of cloth, and that everything save the spermatic cells is destroyed by the strong acid.

TRYPSIN BROTH—AN IDEAL MEDIUM FOR MAKING BLOOD CULTURES

A Preliminary Report*

By R. G. Owen, M.D., F. A. Martin, M.D., and W. G. Pitts,
Detroit, Mich.

WHILE working on the treatment of suppurating wounds, Sir A. E. Wright observed that when the antitryptic activity of the serum was destroyed by the trypsin set free from disintegrating leucocytes, many organisms grew luxuriantly which were incapable of growth in the presence of serum rich in antitryptic power.

Emery further showed that by neutralizing the antitryptic action of serum with trypsin, the blood lost its coagulability, and with this also went a loss of opsonic, complementing, and bactericidal action.

Reasoning from this basis, Douglass and Colebrook decided that the addition of proper amounts of trypsin to beef-tea would furnish an ideal medium for blood cultures, and certain experiments which they made corroborated their views.

*From the Bacteriological Department of the Detroit Clinical Laboratory.

We have been able to confirm their results in the main; and from our experimental and clinical results, can most strongly recommend "Trypsin Beef-Tea" as an ideal medium for making blood cultures.

We have tried various preparations of trypsin in different strengths, and have found the "Liquor Pancreatica" made by the Digestive Ferments Co., of Detroit, Michigan, to be the most satisfactory.

The Digestive Ferments Co. supply this preparation, sterilized by passage through a Berkefeld filter, in 10 c.c. ampules.

This amount should be added to 90 c.c. of sterile beef-tea and 5-10 c.c. of the mixture put in sterile test tubes. The medium must be handled aseptically, as heat destroys the action of the trypsin. When making cultures, add 1 c.c. of blood to each of 5 to 10 tubes of medium.

Trypsin beef-tea, we have found, keeps its strength for at least four weeks. Staphylococci, streptococci, pneumococci, and typhoid bacilli from the blood stream, all show a much earlier and more uniform growth in the trypsin medium than do cultures in plain or glucose beef-tea.

We would greatly appreciate any case reports dealing with the use of this medium in isolating organisms from the blood.

A STANDARD METHOD FOR MAKING UNIFORM COLLOIDAL GOLD SOLUTION

By William K. Trimble, M.D., Kansas City, Mo.

ALL glassware should be rinsed successively with a small amount of freshly distilled water, neutral 95% alcohol, ether and finally heated in a hot air oven.

The water used should be freshly distilled in an all-glass still without rubber connections. Redistilling is not necessary.

The solutions of gold chloride, potassium carbonate and formaldehyde solution are prepared in the usual way and kept in a dark place in well fitting glass-stoppered bottles.

One should have a separate pipette for each solution used in the technic and these should not be used for other laboratory work.

Only chemically pure reagents should be used. In making the 1% formaldehyde solution treat the usual 40% as 100%.

Technic.—Distill 500 c.c. of water into one of the especially cleaned Erlenmeyer flasks and place on an iron tripod. Interpose between the flame and the flask a disc of copper about the thickness of a heavy blotting paper. The object of the copper disc is to permit a more uniform heating of the water and at the same time prevent overheating the solution at points in the bottom of the flask. Insert a clean thermometer into the water and when the temperature has reached 65° C. add at once 5 c.c. of the 2% potassium carbonate solution. Stir, and when the solution has reached 75° C., immediately add 5 c.c. of the 1% gold chloride

solution. Stir and bring the temperature of the solution to 90° C. Remove the flame and begin adding the 1% formaldehyde solution quite a few drops at a time and with constant stirring. It is important not to be hasty in adding the formaldehyde solution. When 3 to 3.5 c.c. have been added, allow considerable time before more is used, then only a few drops at a time. In a short while a perceptible change in the color of the solution will be seen, when no more of the formaldehyde solution should be added. Let the flask remain on the hot copper disc until the solution changes to a bright, clear, deep red color, when the flask is removed and allowed to cool. The color deepens slightly on cooling.

It is not necessary to reheat during or after the addition of the formaldehyde solution, so long as the whole remains at 90° C.

THE PRESERVATION OF ERYTHROCYTES FOR THE WASSERMANN REACTION*

By Stanley P. Reimann, M.D., Cleveland, Ohio.

NUMEROUS methods have been devised for the preservation of erythrocytes for use in the Wassermann reaction and in other immunologic procedures. The time, trouble, and expense of the weekly or semi-weekly trip to the abatoir can be much reduced if a simple and satisfactory method can be utilized which will give results with preserved cells identically the same as results obtained with fresh cells. The present study compares results obtained with fresh cells and cells preserved by the formalin method and by the saccharose-gelatin method of Rous and Turner. For this purpose 200 tests were made with cells preserved by the Rous Turner method and 150 tests with both the Rous Turner and formalin methods. In all cases simultaneous tests were made with cells from 1 to 4 days old kept in the ice chest. Complement and hemolytic amboceptor were titrated separately against each sample of cells used and in the vast majority of cases, the results were identical; differences when found were small.

The formalinized cells were satisfactory for at least three weeks, several specimens for four weeks. The first cells preserved by the gelatin and saccharose solutions were stored in cotton-stoppered vials and hemolysis in sufficient amount to render the cells useless occurred in eighteen days. Those preserved subsequently were placed in sealed ampules and they were satisfactory for from 21 to 25 days.

The discrepancies in the final readings amounted to 6 per cent of the total number but no uniform variation was to be discerned. In some instances the preserved cells gave negative readings and the fresh cells positive readings, and in other instances the readings were reversed. In all these cases, the four antigens used,—two cholesterinized alcoholic extracts of human hearts, one cholesterinized alcoholic extract of ox hearts, and an alcoholic extract of syphilitic

*From the Departments of Pathology and of Dermatology, Western Reserve University.

liver, gave contradictory readings with preserved and with fresh cells. Reference to the clinical histories of many of the patients from whom these sera were obtained gave evidence of treated lues in various stages.

The method of preservation as worked out by Rous and Turner was carried out in the following manner: The blood of a sheep was run from the carotids directly into Locke's solution containing one per cent sodium citrate in the proportion of one part of blood to four parts of solution. The corpuscles were separated by rapid centrifugalization and carefully washed three times in Locke's solution containing 0.25 per cent gelatin. The cells were then placed in a layer not more than 2 mm. in depth and covered with saccharose-Locke solution to a depth of about 2 cm., the ampules were sealed and stored at a temperature of 5° C. and 6° C. Just prior to use the cells were washed once with .85 per cent saline solution to remove the saccharose solution, and proper dilution effected with saline. Strict asepsis was observed.

The cells for formalin preservation were prepared by allowing the sheep's blood to run directly into formalin solution in the proportion of 0.5 c.c. of 40 per cent formaldehyd solution to 400 c.c. of blood. Defibrination was accomplished by shaking with glass beads. The mixture was not disturbed until ready for use, when the cells were washed three times with 0.85 per cent saline solution in the usual way.

THE SOLUTIONS.

Locke-sodium citrate solution.—

Sodium citrate	10	grams
Sodium chlorid	9.2	"
Sodium bicarb	0.05	"
Potassium chlorid	0.1	"
Calcium chlorid	0.1	"
Aq. dest. q.s. ad	1000	c.c.

Locke-gelatin solution.—

Gelatin	2.5	grams
Sodium chlorid	9.2	"
Sodium bicarb	0.05	"
Potassium chlorid	0.1	"
Calcium chlorid	0.1	"
Aq. q.s. ad	1000	c.c.

The Locke and saccharose solutions are sterilized separately and used in the proportion of 2.8 c.c. of the saccharose solution and 7.5 c.c. of the Locke's solution.

Saccharose solution.—

Saccharose	103.0	grams
Aq. q.s. ad	1000	c.c.

Locke's solution.—

Sodium chlorid	9.2	grams
Sodium bicarb	0.05	"
Potassium chlorid	0.1	"
Calcium chlorid	0.1	"
Aq. q.s. ad	1000	c.c.

CONCLUSION.

Under ordinary laboratory conditions sheep erythrocytes for use in the Wassermann reaction can be preserved satisfactorily from 3 to 4 weeks by the formalin method, and for from 21 to 25 days by the Rous-Turner method. The readings obtained differ from those obtained with fresh cells only in so far as some sera produce slightly different results when used with cells from the same specimen of sheep blood.

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A SIMPLIFIED COMPLEMENT FIXATION TEST*

By Norman E. Williamson, M.D., Stockton, Calif.

THE following method is a modification of Noguchi's test, but is much simpler and quicker.

Capillary pipettes are drawn out of small glass tubing. With a light touch of a file a mark is made at the point which 0.06 c.c. of mercury reaches from the end of the capillary tubing. The entire length of the pipette is 12 cm. Blood is drawn from the ear to the mark. This will contain approximately 0.02 c.c. of serum for each tube of the test as used in the Noguchi test, in addition to cells.

Place the small tubes in the rack used for complement fixation and add to the front tube 1.94 c.c. of citrated salt solution. The salt solution is the usual 0.9 per cent. The citrate is added in the proportion of 4 parts to 10,000. It is convenient to keep on hand salt solution containing 1 per cent citrate. Four c.c. of this and 96 c.c. of salt solution will make the right proportion for the tubes. This will prevent the clotting of the 0.06 c.c. of blood used, and does not in any way interfere with the reaction. If salt solution alone be used, the slight clot which forms detracts from the accuracy of the test.

0.06 c.c. of blood is put in the front tube and immediately shaken. The blood is blown out of the pipette using the mouth piece of a blood counting pipette. 1 c.c. is taken from the front tube for the control tube. The pipette is then washed with salt solution, water, alcohol, ether, and dried in the flame of a Bunsen burner. It is now ready for the next patient. Blood is taken from known positive and negative cases for control.

Add 0.1 c.c. of properly diluted Noguchi antigen to the front row of tubes and 0.1 c.c. of salt solution to the back row.

Add 2 units, usually 0.4 c.c. of 10 per cent complement to all tubes. Shake.

Place in water bath at 37° C. for ½ hour.

*From the State Hospital, Stockton, Calif.

Remove and add 3 units of antihuman amboceptor in 1 c.c. of salt solution. (A unit of amboceptor has the same meaning as usual; i. e., the quantity which will hemolyze 1 c.c. of 1 per cent suspension of washed human red cells in 1 hour with 1 unit of complement.)

Place in water bath at 37° C. for 1 hour.

Compare each front tube with its corresponding back tube. Complete inhibition of hemolysis in the front tube with an average amount in the back tube would be + +. Less than this but more than 50 per cent inhibition would be +. More accurate readings can be made with a colorimeter, using a standard in the wedge and comparing each tube with the standard. Tubes must either be allowed to settle after incubation or they can be at once centrifugated and read.

The amount of red cells used in this test is about equal to 1 c.c. of 3 per cent suspension or 3 times that used in the Noguchi test. This is not a disadvantage, as no more will be hemolyzed than the unfixed complement can manage. The hemolysin is always the same, as none is added with the patient's serum. This might occur if serum and cells came from individuals of different groups, unless the cells belong to group 4 of Moss, as I mentioned in a former contribution.

The advantages of this method are obvious. The blood taking is very simple and relieves the patient of much discomfort. No time is lost in getting clear serum. There is no washing of blood except for standardization of reagents. The test can be completed in 2 hours from the time the blood is taken.

I have found the test to be accurate. I have checked it by both Wassermann and Noguchi results.

A MODIFICATION OF GERHARDT'S TEST FOR DIACETIC ACID IN THE URINE*

By Harvey P. Barret, M.D., Charlotte, N. C.

IN spite of its fallacies, Gerhardt's test for diacetic acid in the urine remains the most reliable and probably the most widely used test for the detection of this substance. The method as described by Webster, is as follows: "To 10 c.c. of urine are added a few drops of ten per cent ferric chloride solution. This is best added as long as a precipitate of phosphates occurs, these latter bodies being then filtered off. To the filtrate are added a few drops more of ferric chloride solution when the urine shows a Bordeaux-red color in the presence of diacetic acid." The disadvantages in the actual performance of this test are: the time required to filter off the heavy phosphate precipitate; the uncertainty as to the amount of ferric chloride solution to be added, sometimes requiring a second filtration to remove all the phosphate precipitate; the difficulty in reading, promptly, a negative reaction; and the inability to detect, with certainty, small quantities of diacetic acid.

The following simple modification of Gerhardt's test is suggested: About

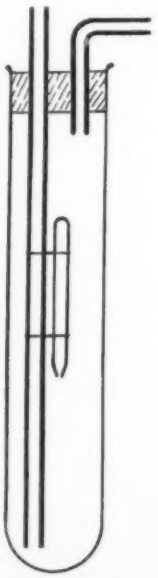
*From the Laboratory of the Charlotte Sanatorium, Charlotte, N. C.

2 c.c. of the urine to be tested are placed in a test tube and an equal quantity of ten per cent ferric chloride solution is allowed to run slowly down the side of the tube. There is thus formed a distinct layer of urine above the reagent. The tube is held at an angle of forty-five degrees. At the point of contact there is a ring of phosphate precipitate, while just below this a Bordeaux-red color is formed if diacetic acid is present. The color appears immediately and tends to diffuse downward into the ferric chloride solution on standing. It may be allowed to stand twenty-four hours, or the tube may be heated as in the original method for differentiating other substances, which give a reaction similar to diacetic acid. The whitish appearance of the phosphate ring serves as a contrast to the red color and makes the reading of a weak reaction much easier. The ferric chloride solution may be first placed in the tube and the urine superimposed or the ferric chloride solution may be run under the urine from a pipette introduced to the bottom of the tube. The best results, however, are obtained by the method as first described.

PREVENTION OF FROTHING IN AERATION METHODS.*

By A. B. Denison, M.D., Cleveland, Ohio.

WHILE working with the urease aeration method for the quantitative determination of urea, we experienced the common difficulty of foaming in the aeration tubes. We finally hit upon the following device, which absolutely controls the foaming and is very economical in the use of antifoam mixture. It also has an added advantage in that the apparatus does not need to be watched at all during a determination:



*From the Medical Research Laboratory, Lakeside Hospital, Cleveland.

Glass tubing of 4 mm. inside diameter is cut into sections about 8 cm. long. One end of each section is sealed off in the flame, and the other is drawn to a point with an opening about 2 mm. in diameter. Some means is then provided of attaching this to the long glass tubing in the aeration tube. Wire clips are easily made that serve the purpose admirably. This short tube is then completely filled with the reagent used to prevent foaming, e. g., amyl alcohol, and it is attached as shown in the figure. The foam rises in the tube until it reaches the capillary tip, when enough alcohol leaves the tube to lower the surface tension sufficiently so as to prevent foam rising higher. Very little alcohol is required. It may be necessary to vary the size of the capillary tip with various chemicals used to prevent foaming.

Experiment shows the amount of ammonia caught in the sealed tube as the alcohol is drawn out is negligible, and does not affect the determination.

This device is applicable to all aeration methods in which foaming occurs.

A small wash bottle (filled with alcohol), having a very fine capillary tip, makes the filling of the sealed tube very simple.